

VERTEBRATE EMBRYOLOGY

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FOURTH EDITION



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To My Wife

Preface to the Fourth Edition

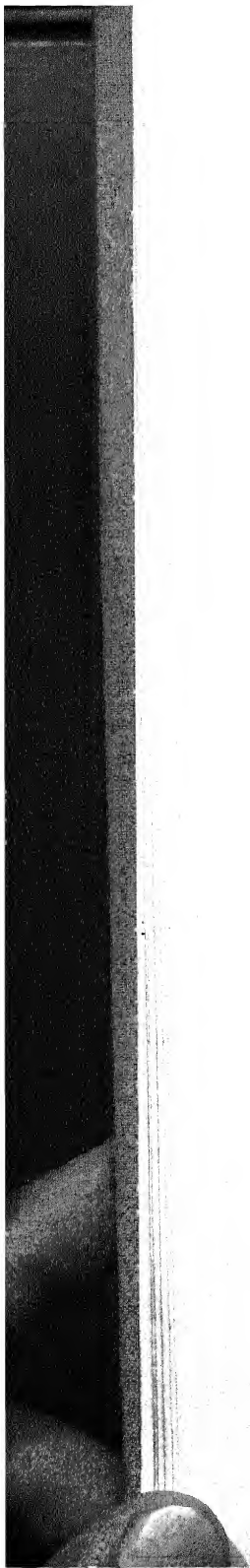
As in previous revisions, the fourth edition of this text does not purport to be a new book. It again frankly retains the fundamental plan and character of the older editions, in that it is primarily descriptive, but with enough experimental results interwoven with the descriptive material to stimulate interest, and to elucidate such principles of development as have been firmly established.

Though not radically altered, the older book has nevertheless been carefully gone over page by page, and, as before, changes have been made wherever it was thought desirable in order to bring the subject matter up to date, to clarify statements, or to correct errors. In some cases, whole pages have been entirely rewritten, and in certain instances, as in the section on maturation of the germ cells, this has involved several successive pages. Mistakes in figures have also been corrected, and in a few cases, as in the diagram of frog gastrulation, the figure has been completely modified and, the writer believes, greatly improved.

Thanks are due to various colleagues who have made suggestions and pointed out errors. Especial gratitude is felt by the author to Dr. Roland Walker for his meticulous notations of errors both large and small, and for his constructive efforts to aid in their correction.

R. S. McE.

Oberlin College,
September, 1956.



Preface to the First Edition

THIS book is designed as an introductory text in Vertebrate Embryology, a work which seems to be justified on the following grounds: The older texts upon this subject, though in many cases excellent, do not cover exactly the field which is now covered in many colleges; these texts, moreover, are becoming somewhat out of date in various details. Among the newer books the best ones tend to do one of two things. Either, in the interest of thoroughness, they confine their attention entirely to one form, e.g., the Chick, or else, for the sake of a broader viewpoint, they deal with a considerable number of animals, but in doing so touch only upon the earlier developmental stages of each. Now it is obvious that there is great value for the student, both in the accuracy gained by the careful intensive study of a single type, and also in the possession of less detailed knowledge of the history of other forms which are nearly related to it. Hence, what has seemed to be needed was a book which would, so far as is possible, make available both these advantages. To meet this need, the major part of the present text comprises a moderately complete account of the development of two typical forms, i.e., the Frog and the Chick, each of which, in the writer's opinion, has special features which justify such treatment. These relatively detailed discussions are then supplemented by chapters which present brief comparisons, not only with the Mammal, but also with certain other significant members of the Vertebrate group. Furthermore, the essentially embryological portion of the book is preceded by an optional introductory chapter dealing with the elements of cytology. Upon this basis the effort throughout the work has been to produce something especially adapted to the requirements of the general student of Zoölogy, as well as to the individual particularly interested in premedical preparation.

As regards certain details concerning the method of handling the topics involved, the following remains to be said. Because of the character of the book, the chapter upon cytology places special emphasis upon the structure, development, and function of the germ cells, with particular reference to nuclear phenomena and their genetic significance. The strictly embryological subject matter is then introduced by a short general discussion of the more fundamental and universal processes of Vertebrate development from the comparative standpoint. This includes a description of the various types of segmentation, gastrulation, and the formation of the rudiments of the nervous system and the

main mesodermal structures. Following these introductory chapters, *Amphioxus* is the first particular type to be considered because of the relatively primitive character of most of its early history. The later development of this animal, i.e., that following the formation of the mesodermal somites, is, however, quite highly specialized in respects which distinguish it from the vast majority of Chordates. As these later stages are without great significance for the general student, they are omitted.

The Frog, as suggested above, is one of the two forms which have been treated at some length. The reasons for such extended consideration in this instance and in that of the Chick are presumably obvious to every Zoölogist. For the sake of the student, however, the value of these animals as subjects of embryological study is indicated in the paragraphs of the text which introduce them. In the case of the Frog, its early history has been presented under the head of certain fairly well recognized stages which lend themselves well to correlation with work in the laboratory. In further pursuance of this method the internal changes have been noted in alternation with those occurring externally. This was done in order that the reader might obtain, so far as possible, a correct idea of the really simultaneous character of these processes. It did not seem feasible, however, in a work of this scope to continue this plan throughout the entire course of development in this animal. The later external changes, therefore, are included under one heading, while the more advanced details of organogeny are described in terms of particular systems.

Following the treatment of the Frog, there has been introduced a very brief account of segmentation and gastrulation in the Teleosts and the Gymnophiona. This has been done despite the realization that in the case of the latter group laboratory consideration will in most cases be impossible. The reason for this is the author's opinion that segmentation and gastrulation in these two classes of animals are extremely valuable in assisting the student to relate these processes in the Frog to those which he is about to study in the Bird. Experience, moreover, has seemed to indicate that the relation of avian and mammalian gastrulation to that in more primitive forms is always particularly difficult for the beginner to grasp, and it is believed, therefore, that any legitimate aid to this end is worth while.

In treating the early stages of the Chick a good deal of stress has been placed upon the method of segmentation and gastrulation. The latter especially has been emphasized because of its peculiar character, and the desirability of making clear its relationship to that in the forms

already studied. The later history of this animal is then presented in daily periods, according to the well-known plan of Foster and Balfour. This has been done because it seems to the writer that at least in a beginning course, this method has certain marked advantages over that of studying the complete embryology of one system at a time. In the first place the Bird lends itself particularly well to treatment by periods, and secondly, the simultaneous development of all the systems is what is actually seen to occur in any animal. This latter fact it would seem well to impress upon the student when possible by the method of presentation. Finally it has appeared not only possible but easier to conduct the class work in correlation with the laboratory when development is studied by periods rather than by systems. It should be noted, nevertheless, that in this book the material has been so arranged that the student can readily follow through the complete growth of any one system if the instructor so desires.

As regards the Mammals, it is felt that the detailed differences between the organogeny of this group and that of the Birds are not, on the whole, of great general biological significance. Of very considerable significance, however, are those unique characteristics of both mother and embryo connected with mammalian gestation. For this reason the discussion in this portion of the text is confined chiefly to the earlier developmental stages, which are treated largely from the comparative standpoint. The subject is introduced by a description of the structure and functions of the adult reproductive organs in the same manner as in the case of preceding forms. This involves the process of ovulation, and in that connection it has seemed worth while to describe briefly the peculiar cyclic phenomena which accompany this process in the mammalian female. Following this, the comparative idea is pursued with particular reference to the development of the extra-embryonic appendages. This is believed to be especially important from an evolutionary viewpoint because it shows how these appendages, already observed in the Chick, have been modified in the various Mammals. This discussion is naturally accompanied by a description of the structure and probable evolution of the placenta. For the general plan of treatment of these latter topics the author frankly acknowledges his indebtedness to Professor Jenkinson's excellent book, *Vertebrate Embryology*.

Concerning bibliographical material, references to the more important literature of each subject are appended to the chapter which concludes consideration of the topic in question. As intimated, it will be quite obvious that these references make no pretense of being exhaus-

tive. Their object is rather merely to point the way to further study for the reader who desires it. This is done, first, because the present volume is intended primarily as a text rather than as a book of reference, and, secondly, because it is felt that the beginner's interest may be more effectively aroused in this manner than by presenting to him at once every reference available. The latter, if desired, can be readily obtained in the more advanced books which are cited.

It is recognized that illustrations constitute an extremely important feature in a text of this character, and the writer has spared no pains in the attempt to make the figures adequate both in number and quality. It will be evident, however, that the majority of them are not original. This is due to the fact that through the kindness of the authors and publishers indicated below, there were made available a large number of excellent illustrations, which it seemed hardly worth while to attempt to improve upon. Nevertheless, in every instance where it was felt that such improvement was possible, or where it appeared that a new figure would be profitable, original drawings have been inserted. Lastly, it remains to be stated in this connection that in the case of all borrowed illustrations, great care has been taken to have the illustration and the terms used in its legend agree with the respective description and terminology in the text. The desirability of this, especially in an elementary book, is obvious; yet, according to the writer's observation, it is a feature which is too frequently overlooked.

In conclusion I desire to express my appreciation of the following favors. To Professor Frank R. Lillie and to Henry Holt and Co., I am indebted for their generous permission to use a large number of figures from Lillie's *Development of the Chick*; to Professor T. H. Morgan, his co-authors, and Henry Holt and Co., for certain illustrations from *The Mechanism of Mendelian Heredity*; to Henry Holt and Co., for numerous figures from Kellicott's *General Embryology* and *Chordate Development*; and to the Delegates and Secretary of the Clarendon Press for a like favor as regards Jenkinson's *Vertebrate Embryology*. It is also a pleasure to acknowledge a similar debt to Professor Morgan and The Columbia University Press for figures from *Heredity and Sex*; to Professor J. Playfair McMurrich and P. Blakiston's Son and Co. for clichés from McMurrich's *Development of the Human Body*; to P. Blakiston's Son and Co. for further clichés from Minot's *Laboratory Text Book of Embryology*; to Messrs. Longmans, Green and Co. for clichés from *Quain's Anatomy*; to Messrs. G. P. Putnam and Co., for permission to use again certain figures from Marshall's *Vertebrate Embryology*,

copied and slightly modified by Kellicott; and to Professor O. Van der Stricht and Dr. T. W. Todd for allowing the use of photomicrographs made in the Anatomical Department of Western Reserve University Medical School from preparations presented to that department by Professor Van der Stricht. In all cases the illustrations thus borrowed are acknowledged in the legends of the figures concerned.

I wish further to express particular gratitude to Professor T. H. Morgan for reading and criticizing the first half of the manuscript; to Professor J. H. McGregor for performing a similar service for the entire book; to Professor M. M. Metcalf for suggestions regarding the earlier chapters; to my wife for assistance with the proof; and to Professor R. G. Harrison for the identification of the frog larvae used in making certain of my original drawings. Especial gratitude is also felt for the constant interest and helpfulness shown by my colleagues, Professors R. A. Budington and C. G. Rogers.

R. S. McE.

OBERLIN COLLEGE,
August 15, 1923.

C O N T E N T S

PART I: THE GERM CELLS AND EARLY

CHAPTER

DEVELOPMENT OF AMPHIOXUS

1. Introduction	2
2. Fertilization and Early Stages in Development	39
3. The Early Development of Amphioxus	75

PART II: THE DEVELOPMENT OF THE FROG

4. The Frog: from the Production of the Germ Cells through Gastrulation	104
5. The Frog: Early or Embryonic Development Subsequent to Gastrulation	147
6. The Frog: Later or Larval Development	169

PART III: THE TELEOSTS AND GYMNOPHIONA

7. The Teleosts and Gymnophiona: their Segmentation and Gastrulation	262
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PART IV: THE DEVELOPMENT OF THE CHICK

8. The Chick: the Adult Reproductive Organs, and the Development of the Egg Previous to Gastrulation	280
9. Gastrulation and Development through the First Day of Incubation	300
10. The Chick: Development during the Second Day of Incubation	332
11. The Chick: Development during the Third Day of Incubation	370
12. The Chick: Development during the Fourth Day of Incubation	395
13. The Chick: Development during the Fifth and Subsequent Days	433

PART V: THE DEVELOPMENT OF THE MAMMAL

14. The Early Development of the Mammal and its Embryonic Appendages	486
15. Development of the Pig to the Ten Millimeter Stage	561
16. The Later Development of the Pig	606
17. The Skeleton, Teeth, Hair, Hoofs and Horns	655
Index	673

PART I

THE GERM CELLS AND EARLY
DEVELOPMENT OF AMPHIOXUS

INTRODUCTION

IT has long been an axiom with biologists that all organisms consist either of single cells or of cell aggregations, often with the addition of various cellular products. It is also well known that even in the case of multicellular animals or plants, each individual starts from a single cell. This cell may be one that has recently fused with another in the process called fertilization, or it may develop without such fusion by a process called parthenogenesis. The latter is a natural procedure in some instances and may be artificially induced in others.

With the foregoing facts in mind it may then be stated that the development of any multicellular animal or plant involves three fundamental processes which go on more or less coincidentally. These processes are: The increase in cell numbers by cell growth and division (usually mitotic); the differentiation of the cells and sometimes their products into various tissues; the arrangement of these cells and tissues to constitute parts and organs. It is therefore the study of these processes which comprises embryology. Stated thus baldly and reduced, so to speak, to its lowest terms, the subject may appear rather dry and prosaic. Such, however, is furthest from the truth for anyone with any real interest in living things, and in the problems of existence in general. For there is no more astounding and fascinating drama which one may view than to watch the development of certain eggs. This is particularly true of relatively small transparent ova which it is literally possible to see through in the living state, such as those of many of the Invertebrates, like Sea Urchins or Molluscs, and even some Vertebrates, like many Fish. Here one may observe under the microscope the active division of the cells and their gradual differentiation and rearrangement. Thus in certain rapidly developing forms, there may be seen in a few hours the transformation of an apparently structureless blob of jelly into a clearly recognizable and relatively complicated organism. Careful and accurate descriptions of these and many other cases more difficult to observe have been recorded for a long time, and this constitutes descriptive embryology. It was inevitable, however, that after observing this veritably magical performance man should begin to inquire how it was done, and

this inquiry has led to the growing and very active field of experimental embryology. Hence at first by relatively crude acts of interference with normal development, and later by more cleverly planned procedures it was and is being sought to analyze the fundamental processes involved. As in the analysis of all life phenomena the goal has constantly been to reduce them to physico-chemical terms; and though this end is by no means attained, workers everywhere are constantly pressing toward it. Hence, though the primary aim of this book is to present a description of normal embryological phenomena, opportunity will frequently be taken to indicate how experiment has helped to throw light on many of the basic mechanisms concerned.

It has been stated that development starts from a cell and that cells constitute the units or building blocks of which living structures are made. We might therefore spend some time in a discussion of cell structure and physiology. For the purposes of this book, however, it is assumed that the student is already familiar with this subject, and with the phenomenon of normal cell division or mitosis. We shall therefore omit further reference to this matter. It does, however, seem desirable to make some comment as to the origin and history of the germ cells. Let us then begin with this topic.

THE GONADS AND THE GERM CELLS

The germ cells or gametes are certain cells in which both cytoplasm and nucleus are specialized for the purpose of reproduction. They are thus distinguished from body or *somatic* cells which are specialized for other functions in the life of the organism. Before considering the detailed development of the germ cells it will first be necessary to give a brief history and description of the organs in which they are finally located.

THE GONADS

The germ cells of the adult occur in organs known as gonads, the female gonad being termed the ovary, and the male gonad, the testis. In most true Vertebrates these are paired structures, and in the same individual both members of the pair are normally of the same sex. In their earliest condition both ovaries and testes appear alike, as a pair of ridges (the *genital ridges*) consisting largely of thickened coelomic epithelium (the *germinal epithelium*). Beneath this epithelium there occur a mass of loose mesodermal cells known as mesenchyme. Pres-

ently these cells give rise to real connective tissue which soon increases and constitutes the supporting element of the organ, termed the *medullary tissue* or *stroma*. Each genital ridge lies along the back on either side of the dorsal mesentery of the gut between it and the embryonic excretory organ. Within the germinal epithelium there presently appear

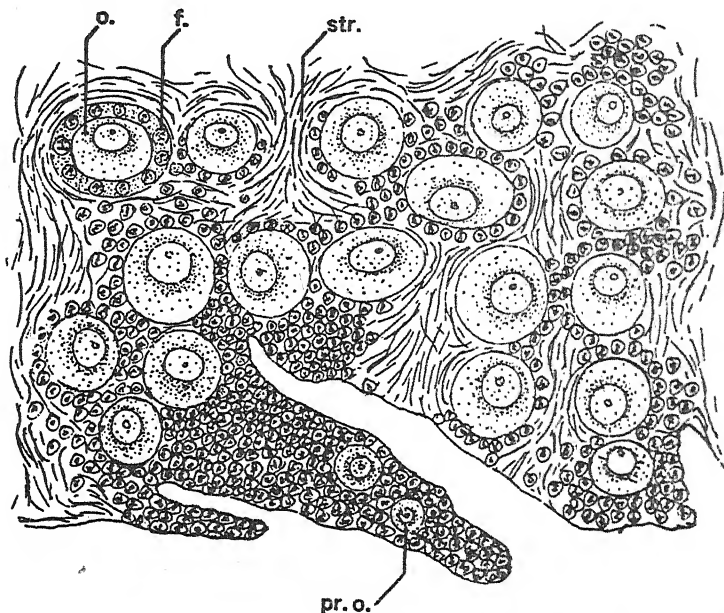


Fig. 1. — Cross section of the ovary of a fledgling of *Numenius arcuatus* 3-4 days old. From Lillie after Hoffmann. The region of the germinal epithelium is toward the bottom of the figure. *f.* Follicle. *o.* A very young ovum around which the epithelial cells have formed a definite follicle. *str.* Stroma. *pr.o.* Primitive ovum within a portion of the germinal epithelium.

certain cells which are often distinguishable from their fellows by their larger size and also by their relatively larger nuclei. These are the primitive or *primordial germ cells* in which sex differentiation, at least as regards the cytoplasm, is not yet apparent. The origin and later development of these cells will be discussed after completing our description of the gonads.

The Ovary. — In the case of the ovary, as the germinal epithelium gradually increases in thickness it is in some instances divided by the stroma into columns or strands termed the *ovigerous cords*. In any event, during the course of growth, groups or nests of the epithelial cells, each containing a primitive ovum, become scattered about

throughout the connective tissue. Each germ cell then proceeds to develop as such, while the epithelial cells which surround it, known as its *follicle* (Fig. 1), serve to convey it nutriment.

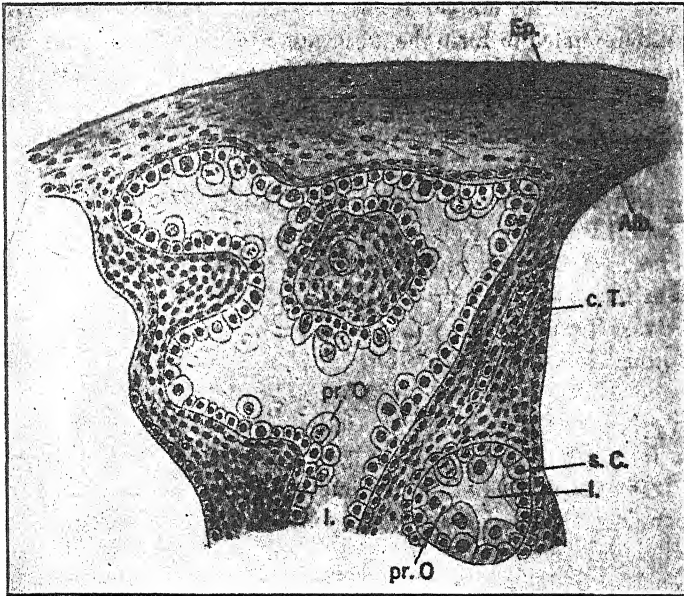


Fig. 2.— Cross section through the periphery of the testis of a just hatched Chick. From Lillie (*Development of the Chick*). After Semon. The sexual cords have acquired a lumen, and the walls of the canals thus formed are lined within by the spermatogonia. Next to the latter come a layer of supporting or Sertoli cells, and outside of these a thin layer of connective tissue, the theca (not labeled). The remaining connective tissue (stroma) lying between the sexual cords (now seminiferous tubules) connects at the periphery of the testis with the special layer of connective tissue (albuginea) which covers the entire organ beneath the thin outermost layer of coelomic epithelium.

Alb. Albuginea. *c.T.* Connective tissue of the stroma, or septulae testis. *Ep.* Remains of the germinal epithelium now forming the outermost or serous covering of the testis. *l.* Lumen of the sexual cords. *pr.O.* Spermatogonia. *s.C.* Sexual cord, lined by supporting cells and spermatogonia.

The Testis.— Within the young gonad which is to become a testis there develop throughout the stroma, strands of tissue, in this case termed *sexual cords*. Though their origin in some instances is doubtful, they apparently arise, like the ovigerous cords, from the germinal epithelium. Whatever their origin, however, they presently become filled with the germ cells which seem to migrate into them. These cords then

become tubular, and the tubes are lined by the germ cells, either arranged in layers or enclosed in cysts (some Amphibia). Certain of the cells constituting the walls of the cysts, or tubes, as the case may be, are homologous in function to the follicle cells of the ovary, i.e., they bring nutriment to the growing germ cells. These nourishing cells in this case are often termed *supporting* or *Sertoli cells*. Externally each tube is covered with a thin layer of connective tissue termed the *theca*, and the whole is known as a seminiferous tubule (Fig. 2).

A more detailed description of the development and structure of a typical vertebrate ovary and testis will be found in our treatment of this subject in connection with the Chick. Likewise short discussions of these organs are included in the accounts of the other animals to be studied. With this as an introduction the student is now prepared for a description of the history of the actual germ cells.

THE GERM CELLS

The Origin of the Primordial Germ Cells.—There have been two theories regarding the origin of the germ cells. It was originally believed that they arose through the modification of certain cells of the germinal epithelium. In the earlier part of the century, however, it was discovered that in some animals, at least, the primordial cells were not first seen in the germinal epithelium at all, but were discernible as far away as various parts of the gut wall. From thence they were seen by some to migrate to the gonad through the mesentery of the gut, as in the Turtle and Gar-pike (B. Allen, '06, Fig. 3), or to be moved thither by shifting of parts due to growth as in the Amphibia (Humphrey, '25), or to be carried by the blood stream as in the Chick (Goldsmith, '28).

While evidence for this sort of thing has continued to accumulate, other observers have questioned the ultimate fate of these migrating cells. In many cases it is claimed that such cells are not the ones which form the actual or definitive germ cells. It is asserted on the contrary that the so-called primordial cells degenerate, and that the definitive germ cells arise later by the transformation of indifferent epithelial cells as was originally supposed. This is said to be the case for the Rat by Hargitt ('25, '30), for the Cat by Sneider ('40), for the Opossum by Everett ('42), for the Guinea Pig by Bookhout ('45), and in various other cases. Also in some instances, there are opposing views concerning the same animal as in the case of the Cat in which Kingsbury ('38) claims, contrary to Sneider, that all definitive germ cells come from the primordial ones.

It appears too that the situation may vary in different animals since Everett ('43) thinks that in the Mouse, contrary to his view regarding the Opossum, the primordial cells furnish all the definitive germ cells. Thus it is evident that this question is still an open one, and hence subject to continued research. The reason for reference to it here is that much of this interest in the origin of germ cells in Vertebrates stems

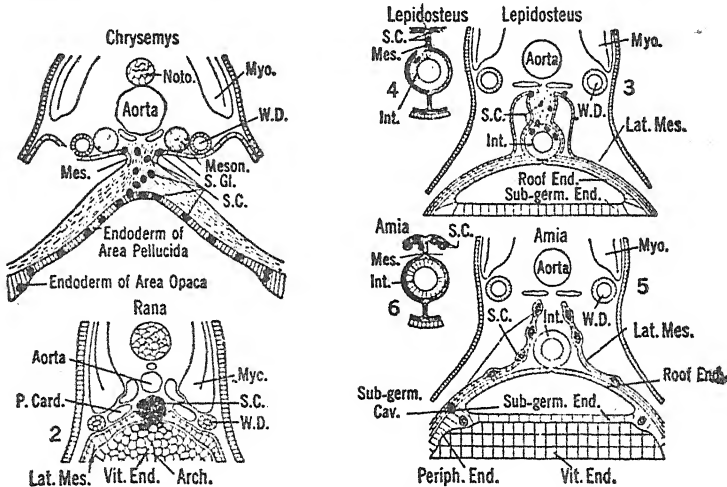


Fig. 3. — From Morgan (*Heredity and Sex*. Published and copyrighted by the Columbia University Press). After Allen. Origin of germ-cells in certain Vertebrates, viz., Turtle (*Chrysemys*), Frog (*Rana*), Gar-pike (*Lepidosteus*), and Bow-fin (*Amia*). The germ-cells are seen migrating from the digestive tract (endoderm). End. Endoderm in various localities. Int. Intestine. S.C. Sex (germ) cells. S.gl. Region of the gonads.

from certain well-known cases of apparently very early origin of these cells in some of the Invertebrates, e.g., the Coelenterates (Weismann, '83) and *Ascaris* (Boveri, '10). These cases in turn were long used to bolster the famous Weismannian theory of the fundamental separateness of the germ plasm and somatoplasm, and also the correlated theory by that author concerning the mechanism of development. Modern genetical and experimental embryological research has pretty much outmoded Weismann's notions as to the nature of the germ cells and the mechanism of development in their original form. The actual source of the germ cells, however, is still obviously a subject of considerable interest to biologists. Let us now turn to a consideration of the structure and development or *maturation* of a typical female and typical male germ cell.

The Ovum.—The fully developed female germ cell is termed the *ovum*. The ova of different Vertebrates vary widely in size, in the amount and arrangement of their deutoplasm, and in their coverings. They are uniform, however, in their relatively large size and inertness as compared with the male reproductive cell (Fig. 4). They also resemble both the latter and each other in one particular, i.e., the behavior of their chromatin. This latter point involves a rather complicated aspect of maturation termed *meiosis* which comprises two special cell divisions, the meiotic divisions, sometimes known simply as the maturation divisions.

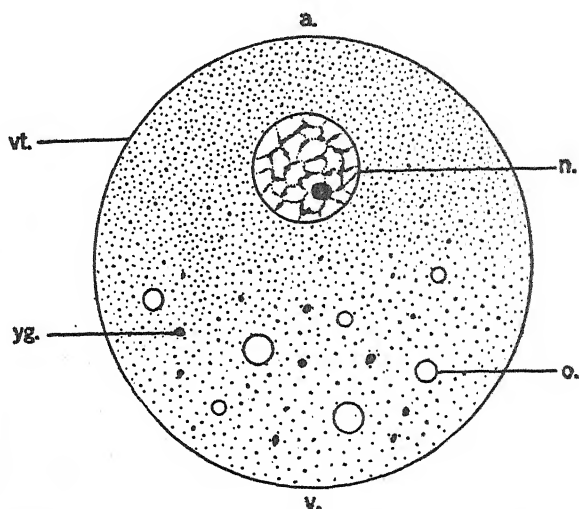


Fig. 4.—Generalized diagram of a slightly telolecithal egg ready for fertilization. The only membrane represented here is the vitelline. *a.* Animal pole. *n.* The nucleus containing a nucleolus and a linin network along the fibers of which chromatin appears. *o.* An oil vacuole. *v.* Vegetal pole. *vt.* Vitelline membrane. *yg.* A yolk granule.

Inasmuch as these divisions are not only complicated, but also of great significance, they will be considered later under a separate heading. The other features of maturation in an ovum and then in a spermatozoon will now be discussed.

It has already been noted that the primordial germ cells which migrate into the germinal epithelium are not readily distinguishable as to sex, at least as regards their cytoplasmic morphology. Their male or female character becomes apparent, however, as the gonad develops

and they become distributed through the stroma of the ovary, or take their places in seminiferous tubules as the case may be.

In the former instance which is now under consideration the young female germ cells in and near the epithelium proceed for a time to multiply quite rapidly. They do this by means of typical mitotic divisions, and during the process are known as *oögonia*. This stage of

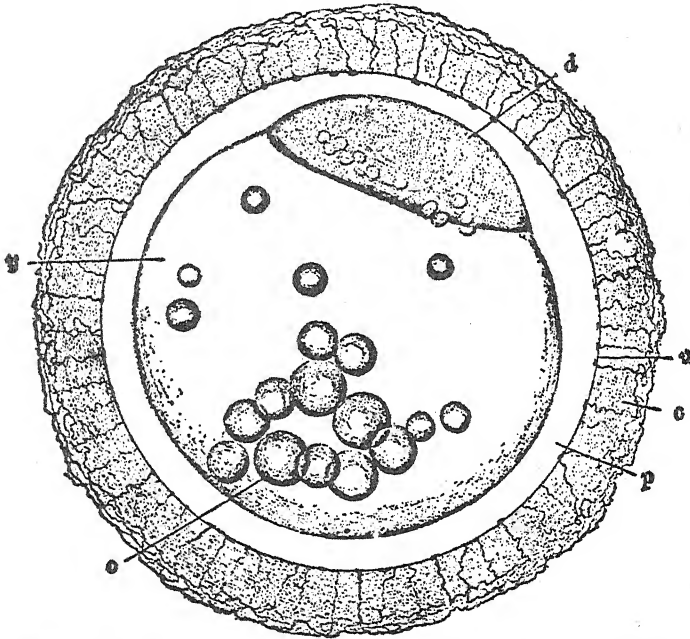


Fig. 5. — Egg of the Teleost, *Fundulus heteroclitus*. From Kellicott (*General Embryology*). Total view, about an hour after fertilization.

c. Chorion. d. Protoplasmic germ disc or blastodisc. o. Oil vacuoles. p. Perivitelline space. v. Vitelline membrane. y. Yolk.

simple multiplication usually continues at least until the time of birth or hatching of the animal in which they are contained. According to most accounts the multiplication of cells then ceases, so that at this time the animal in question contains as many — though only partially grown — ova as it will ever have.

The next period is one of growth during which the cell becomes surrounded by its follicle, and is termed an *oöcyte*.

The Nucleus. — The nucleus during this second period enlarges greatly, and is known as the *germinal vesicle*. It is relatively clear, though it usually contains a fine reticulum, and may possess one or

more conspicuous nucleoli. The latter may be of either the plasmosome or the karyosome type or both, and their significance is not well understood. It probably varies in different cases. At the end, and also sometimes at the beginning of the growth period, certain changes occur in the nucleus which are connected with meiosis. These will be described below.

The Cytoplasm. — Meantime the cytoplasm is increasing considerably in bulk, chiefly as a result in many cases of the accumulation of deutoplasm or yolk. This substance usually first appears in the shape of granules and droplets. Later it assumes various forms and contains a variety of chemical substances, consisting in general of proteids, nucleo-albumins, fats, carbohydrates, and certain salts. Not only does the composition of the yolk vary, but also its amount and distribution. Thus where the amount of deutoplasm is large the oöcyte becomes relatively enormous as in the eggs of Birds and some Fish. In such forms the yolk comes to be situated on one side of the ovum — the *vegetal pole*, whereas the remaining cytoplasm containing the nucleus occupies a greater or less part of the opposite side, or *animal pole*. Ova of this type are said to be *telolecithal*, and in those instances where this arrangement is most marked the relatively yolkless cytoplasmic cap at the animal pole is called the *blastodisc* (Fig. 5). In other ova, such as those of the Mammal, there is relatively little yolk and this is scattered throughout the cytoplasm. An egg of this type is termed *homolecithal*.

The manner in which the yolk originates and grows is of some interest. The actual new material for its formation is of course supplied from without, probably through the medium of the follicle cells. The organization of this material into yolk, however, often seems to take place in connection with a certain body known as a *yolk-nucleus-complex*. The nature and even the exact origin of this body is rather uncertain, and indeed seems to vary in different cases. Frequently, however, it is seen near the true nucleus as a clear spheroidal mass, similar to if not identical with an idiozome,¹ containing a granule or granules (centrioles), and surrounded by a layer (*pallial layer*) consisting partly of Golgi bodies and mitochondria. Whatever its nature when present it seems to exercise some influence over the building up of the nutritive material.

The Central Body. — Concerning this body in the oöcyte there is considerable question. In some eggs, as just indicated, the oögonial divi-

¹ This is a special term applied to the centrosome during certain stages in the development of the germ cells.

sion-center appears to persist for a time as a part of the yolk-nucleus-complex. Before the yolk has finished forming, however, this complex generally disappears, and with it the division-center also usually vanishes. At the time of meiosis a new center forms, apparently in connection with a new (?) centriole, the origin of the latter in these cases being uncertain.

The Egg Membranes. — Following growth the oöcyte, or ovum, as it may now be called, is often surrounded by as many as three different types of coverings, whose character and development are as follows. The first of these is a thin envelope immediately surrounding the egg, termed the *vitelline membrane*. It is doubtful in the eggs of many Vertebrates whether or not this covering is really present. When it is present, however, it is characterized by the fact that it is a secretion from the ovum itself. The second covering is the *chorion*, which is secreted by the follicle cells. It varies much in structure and again may be entirely lacking, as is probably the case in the Chick. Finally there are frequently one or more *tertiary coverings*. These may be jelly-like as in the Frog, or one soft and the other calcareous as in the Bird. When present they are always secreted by some portion of the oviduct through which the egg must pass on its way to the exterior.

The Spermatozoön. — The mature male germ cell is called the *spermatozoön*. In general it is characterized by its extremely minute size, its lack of any nutrient material within itself, and its equipment for active locomotion through a semi-fluid medium. More particularly such a typical sperm consists of the following main parts (Fig. 6):

I. The Head. — This is chiefly composed of concentrated chromatin enclosed in a thin envelope of cytoplasm. It varies greatly in shape in different animals, but is often a more or less ovoid disc. To its anterior end is attached a tip, usually rather pointed, but also subject to much variation in form. It is the *acrosome* or *perforatorium*, apparently derived from a part of the centrosome or idiozome. Thus the head may be said to consist essentially of the nucleus and a very little cytoplasm.

II. The Middle Piece. — This has long been a convenient descriptive term rather than an accurate designation of a part which is truly homologous in different forms, and is in general the region immediately posterior to the head. According to Bowen ('24), however, this part may be more accurately described as that portion of the spermatozoön which is composed of the following materials: cytoplasm, mitochondria, the axial filament, and a centriole or centrioles, to one of which the filament is attached. Of these items, moreover, the mitochondria and

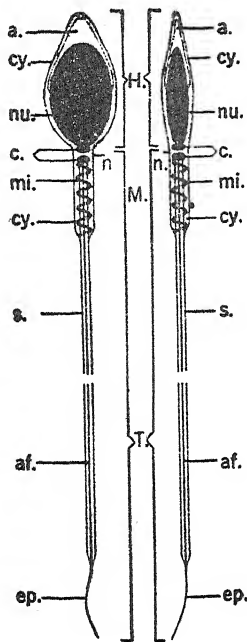


Fig. 6. — A diagram of a generalized flagellate spermatozoon based on the Mammalian type, showing the flat side of the head and also its edge.

H. Head. M. Middle piece. T. Tail. a. Acrosome. af. Axial filament. c. Centrosome. cy. Cytoplasm forming an envelope for the head and middle piece. ep. End piece. mi. Mitochondria arranged in the form of a spiral thread. s. Sheath of unknown origin and constitution covering the axial filament of the main piece of the tail, and extending up inside the cytoplasm of the middle piece. n. Neck.

study of the ovum, and it was noted that during this early period their appearance is practically alike in both sexes. Thus no further account of this stage is necessary in describing the history of the male cell.

centrioles are supposed to be confined to the middle piece, thus defining it. Sometimes at its anterior end is a short clear region of the middle piece attaching it to the head. It is termed the *neck*, and when it exists one or more of the centrioles lies in it.

III. *The Tail or Flagellum.* — Continuing with the definitions of Bowen, this part of the sperm extends posteriorly from the point where the cytoplasm and mitochondria of the middle piece end. It thus consists of that region of the axial filament which though lacking these coverings is nevertheless enveloped by a sheath, plus a short final portion of naked filament. The sheathed region is termed the *main piece*, and the naked filament the *end piece*. The former, along with the middle piece, may also possess a fin-like membrane which is supposed to arise from the axial filament. It should be noted that according to this description some sperm, e.g., those of the Urodeles, have no main piece, the middle piece extending all the way to the end piece.

It must now be added that though the chief features thus described may be regarded as typical of spermatozoa in general, there are numerous, and sometimes quite bizarre, variations. Indeed in certain cases even the characteristic flagellum is lacking, and the cell depends upon amoeboid movements for its locomotion. A suggestion of the varieties of forms which occur is indicated in Figure 7.

With this idea of the general structure of a sperm in mind, it is now possible to consider the stages through which such a cell passes in its development or maturation. The primordial germ cells have already been described in the

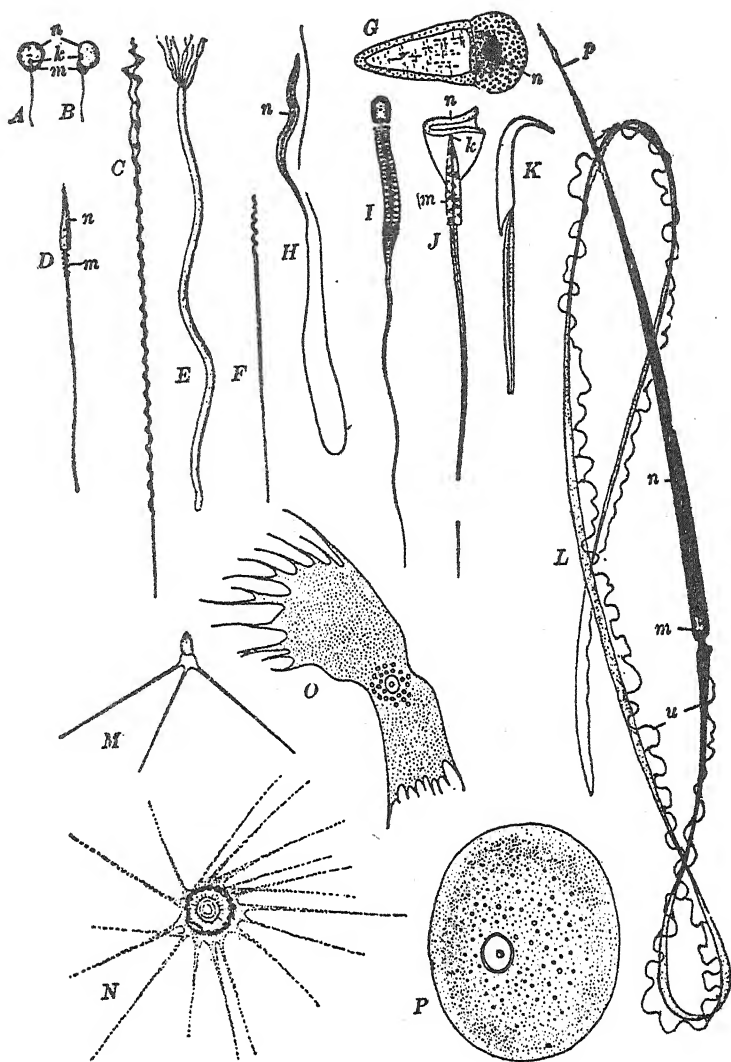


Fig. 7.—Various types of spermatozoa. From Kellicott (*General Embryology*). A, B. The Teleost, *Leuciscus* (Ballowitz). C, D. The Birds, *Phyllopus* and *Tadorna* (Ballowitz). E, F. Two forms of the sperm of the Snail, *Paludina* (von Brunn). G. The Nematode *Ascaris* (Van Beneden). H. The Annulate, *Myzostoma* (Wheeler). I. The Bat, *Vesperugo* (Ballowitz). J. The Opossum, *Didelphys* (Wilson). K. The Rat (Wilson). L. The Urodele, *Amphiuma* (McGregor). M. The Crustacean, *Ethusa* (Grobber). N. The Crustacean, *Inachus* (Grobber). O. The Crustacean, *Sida* (Weismann). P. The Crustacean, *Bythotrephes* (Weismann).

k. End knob. m. Middle piece. n. Nucleus. p. Perforatorium. u. Undulatory membrane. Not drawn to same scale. A-F, I-K, from Wilson.

By the time the male germ cells have become located in the seminiferous tubules, they have become clearly distinguishable as such. They then enter upon a period of multiplication in which they are known as *spermatogonia*. This stage corresponds in all essentials to the similar period of multiplication of the young ova (*oögonia*).

Following this stage is a time of growth which also corresponds to a period of like change among the ova (*oöcytes*). The cells at this time are therefore called *spermatocytes*. In this case, however, the growth, though noticeable, is naturally much less marked than was observed in the *oöcytes*, and there is, of course, no accumulation of yolk. The nucleus, nevertheless, goes through processes very similar to those which characterize the ovum at this period, at the close of which it undergoes meiotic divisions. Although these divisions are fundamentally the same as those of the *oöcyte*, they differ in certain important details which will be considered more fully when that topic is discussed.

Other Differences between the Development of the Sperm and the Ovum. — It will be recalled that in the case of the ovum the end of the growth period found it practically completed. This, however, is one of the points in which the spermatocyte differs strikingly from the female cell. After meiosis the products of the second division are called *spermatids*, and instead of being complete they are just ready to enter upon their remarkable metamorphosis into the highly specialized spermatozoa. This process varies considerably in different animals as regards its details, particularly with respect to the exact method of formation of the middle piece and tail. Indeed there is still so much difference of opinion on the matter, that it seems inadvisable in a text of this type to attempt a description beyond an indication of the general constitution of each of the main parts as already stated. The student interested in the details of metamorphosis as it has been described in a particular form is referred to the account of the process in the seal by J. R. Oliver ('13).

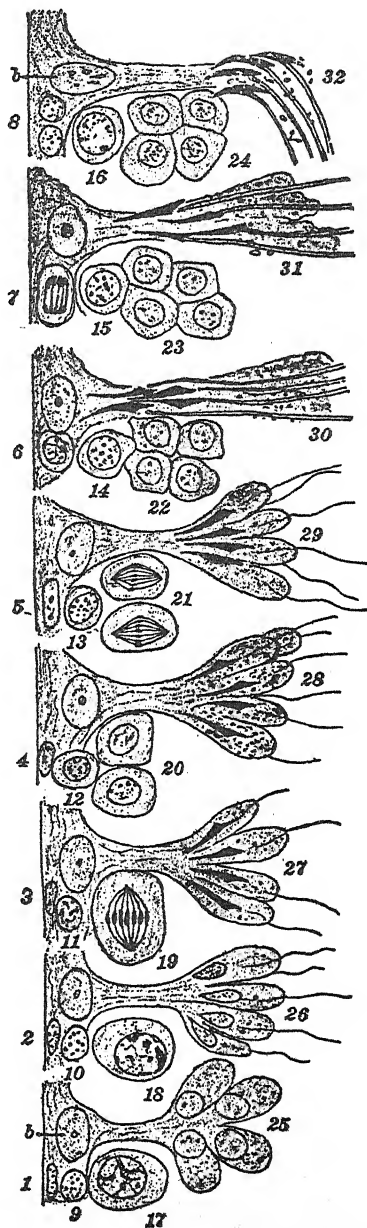
Two further differences between the history of the egg and sperm may finally be noted as follows: One of these is the fact that the multiplication of *spermatogonia* does not cease during the sexual life of the animal. This of course is correlated with the almost continuous production of vast numbers of spermatozoa in comparison with the relatively much smaller production of eggs. As a result of this condition, all the various stages of developing sperm are always to be found in the seminiferous tubules. Where there are no cysts, the youngest cells occur next

to the epithelium, and the older ones successively nearer the central lumen. Where there are cysts, on the other hand, any one, at a given time, usually contains only cells of one stage. In view of the very great number of spermatozoa thus produced, there is perhaps even more question in their case than in the case of the ova, whether all are derived from the original primordial germ cells. Instead it seems probable that some at least arise directly from the division of apparently indifferent epithelial cells.

The second difference is the arrangement of the developing sperm relative to their source of nutriment. It has already been indicated that the cells (*Sertoli cells*) which furnish this do not, except sometimes in the earliest stages, surround each spermatozoön. Instead they form the lining to either a tubule or cyst containing many such germ cells. Then as the development of these

Fig. 8. — Diagrammatic outline of the spermatogenesis of the Rat in thirty-two stages. From Kellicott (*General Embryology*). After v. Ebner. Theca of tubule toward the left. Lumen of the seminiferous tubule toward the right.

1-8. Period of multiplication (the number of cell generations is actually very large). 9-18. Period of growth. 19-24. Period of meiosis. 25-32. Period of metamorphosis. *b*. Basal cells or Sertoli cells. 1-16. Spermatogonia. 17, 18. Primary spermatocytes preparing for division. 19. First spermatocyte division. 20. Secondary spermatocytes. 21. Secondary spermatocyte division. 22-25. Spermatids. 26-31. Transformation of spermatids. 32. Fully formed spermatozoa.



cells proceeds, they become arranged in bundles, all the heads of one bundle becoming imbedded in a single nutrient cell. When the sperm are mature the cyst wall, if there be one, breaks so that their tails project freely into the lumen of the tubule. At the same time the spermatozoa become loosened from the Sertoli cells and are thus ready to be released into the above mentioned lumen (Fig. 8).

MEIOSIS

It is now necessary to return to the consideration of a process which is common to both ovum and sperm, i.e., meiosis. As has already been indicated, the phenomenon is a rather complicated one. Furthermore, it varies somewhat in different animals, and the exact meanings of some of its stages are still in considerable doubt. For the sake of necessary brevity and clearness, therefore, it will be necessary to limit rather sharply the varieties described, and the possible interpretations of which their stages are susceptible.² Also, inasmuch as there are differences in the behavior of the ovum and sperm, it will be necessary to describe them separately. The male germ cell will be considered first.

Meiosis in the Spermatocyte.

I. The Leptotene Stage. — Shortly after the last spermatogonial division, the chromatin of the enlarging nucleus arranges itself in *spireme* or *leptotene* threads (Fig. 9). These threads are relatively very fine, and appear as a tangled maze in which it is difficult or impossible to determine where any particular thread begins or ends. This often leads to the impression that the threads consist of a continuous network, but this is probably not so. Rather, the most favorable cases indicate that this network really is composed of the thread-like components of the chromosomes known as *chromonemata* (singular *chromonema*) (Figs. 9 [2], 11, I). It is, of course, difficult to determine their exact number, but at this stage there is probably one representing each chromosome, and the number would be the same as that of the chromosomes in the somatic nuclei of the organism concerned.

II. The Synaptene Stage. — At this point it should be recalled that the somatic chromosomes of most organisms, with the exception of one chromosome to be noted later, occur in pairs. The members of a given pair appear alike, and were derived, respectively, one from each parent of the organism in question. Such a pair of chromosomes are called

² For a full discussion of this subject with references to the complete literature the student is referred to *The Cell in Development and Heredity* by E. B. Wilson.

homologous chromosomes as contrasted with a pair produced by mitotic division of a single chromosome, and known as *sister chromosomes*. It then happens that during this stage the chromonemata come to lie side by side in pairs which are thought to represent pairs of homologous chromosomes. Usually these chromonemata converge to the nuclear membrane on the side nearest the centrosome, and extend thence toward the other side of the nucleus (Fig. 9 [4]; Fig. 11, II). Presently the members of the pairs begin to fuse or synapse. If this is the correct interpretation the number of pairs should be just half the somatic number of chromosomes. Unfortunately, however, the threads or chromonemata in this stage are still so fine and tangled that they give only the general impression described above, and it is impossible to determine their number exactly.

Even so, in instances where the pairs of threads are well lined up with their ends toward one pole, a fairly close count can be made; in such cases the results confirm the interpretation indicated. Another type of synaptene occurs in some animals and many plants which is termed *synizesis* or *contraction*. Here the leptotene threads or chromonemata become drawn into a tangled mass, usually somewhat to one side of the nucleus. In this type of synaptene the side by side pairing of the threads is much less clear; yet even here there is some evidence that it is occurring as the contraction into the mass begins, and this is generally assumed to be the case. Sometimes, also, the contraction is not so complete as to obscure the fundamental nature of the process. Whichever appearance this stage may have, there is plenty of indirect proof that a close union of the homologous members of chromosomal pairs is occurring here, and hence the name *synapsis* or *fusion* (Fig. 9 [4-5]; Fig. 11, II, IIa).

III. The Pachytene Stage. — In this stage the threads appear much thicker and often somewhat fuzzy (Fig. 9 [6-7]; Fig. 11, III). They are also obviously fewer in number than in the leptotene, and though an accurate count is again difficult, the number at this time appears to be about half that of the chromosomes in somatic cells. Indeed according to the interpretation generally accepted and here given, this number is exactly half, except for the possible presence of the one odd chromosome to be mentioned later; this has been brought about by the more or less complete fusion of the paired threads of the synaptene. This half number of chromonemata, or of chromosomes, of which they are the equivalents, is known as the *haploid* number, as compared to the number formed in the somatic cells and termed the *diploid* number. It should

be noted, however, that the reduction here indicated is not really a genuine reduction since all the threads are still present in a fused condition. The true reduction comes later. This is emphasized by the fact that in some cases, as in the Orthoptera, for instance, there is always, in

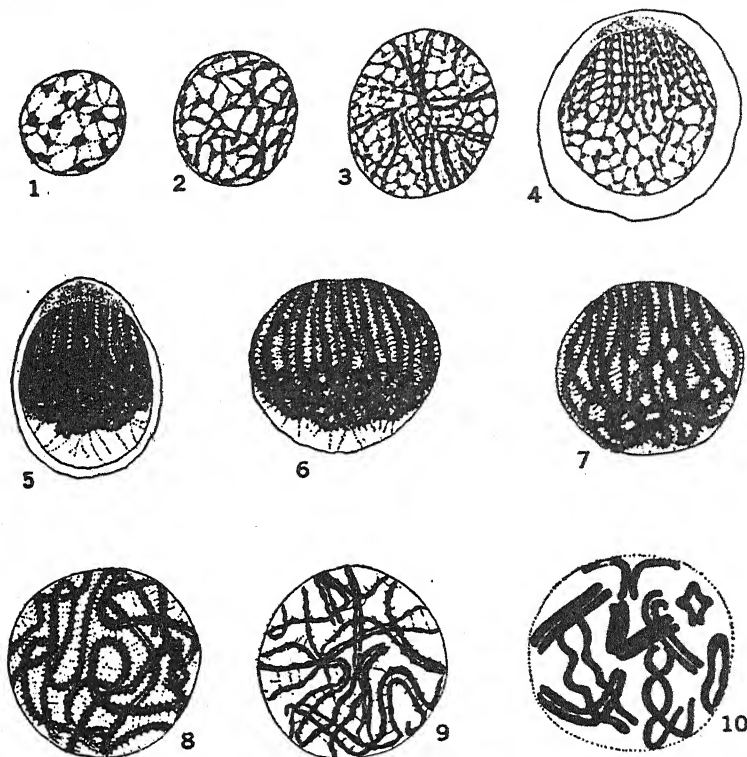


Fig. 9. — Prophases of the heterotype division in the male Axolotl. From Jenkinson (*Vertebrate Embryology*).

1. Nucleus of spermatogonium or young spermatocyte. 2. Early leptotene. 3. Transition to synaptene. 4. Synaptene with the double filaments converging toward the centrosome. 5. Partial synizesis or contraction figure. 6, 7. Pachytene. 8. Early diplotene. 9. Later diplotene. 10. Heterotypic chromosomes with disappearing nuclear membrane and with one figure showing its quadripartite character.

properly stained preparations, a slight indication of the duality of the fused threads.

IV. The Diplotene Stage. — Following the pachytene stage the chromatin threads no longer converge toward one pole, and again appear definitely double. Indeed, especially toward the latter part of this stage, each pair of chromonemata may appear fairly clearly quadripartite, at

which point each one of the four threads is called a *chromatid*, and the group of four is called a *tetrad* (Fig. 9, [9-10]; Fig. 11, IV, IVa). This quadripartite condition is due to the fact that sometime during the pachytene or early diplotene each chromonema of an homologous pair has duplicated itself to form a sister thread. At the same time the four chromatids in each tetrad have become twisted about one another in a

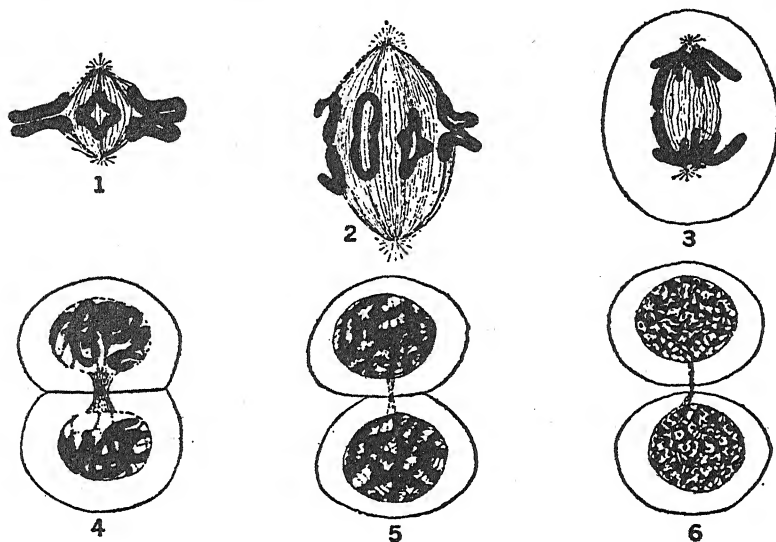


Fig. 10. — First meiotic division in the male. 2. Salamander, the remainder Axolotl. From Jenkinson (*Vertebrate Embryology*). 1, 2. The heterotypic chromosomes on the spindle (metaphase). 3. Anaphase. 4, 5. Telophase. 6. Resting nuclei. 4-6. Cell-division into two secondary spermatocytes.

peculiar way to be explained later, this twisted condition being called *strepsinema* (Figs. 9 [9]; 11, IV). On the basis of the four-part situation just described one might ask why this stage is termed diplotene, meaning double thread. It is because, though the groups may be quadripartite, one of the lines of separation is usually much more evident than the other, and it is along this line that the first meiotic division occurs.

It used to be thought of considerable interest, whether this line represents a separation of the formerly synapsed homologues, or whether it represents a new line of separation between duplicated sister chromonemata, now chromatids. If it is the former, the first meiotic division is said to be *reductional* because it appears to separate the original homologous members of chromosomal pairs. The second division, then, must

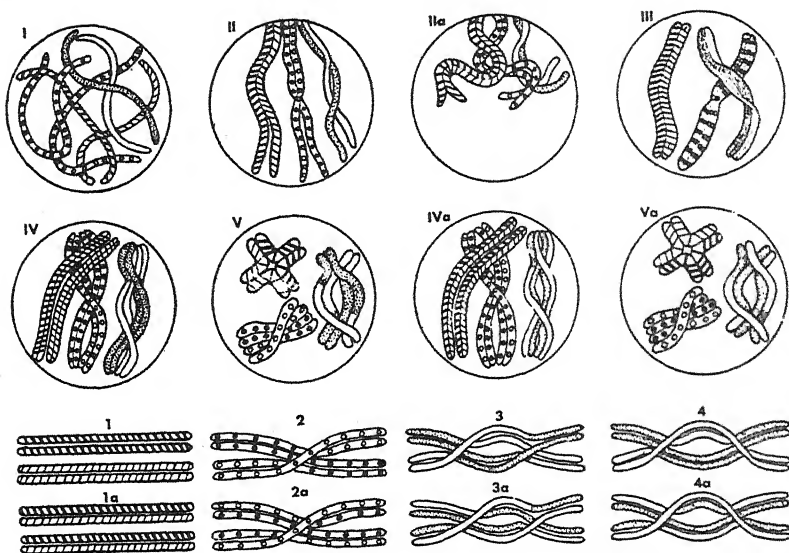


Fig. 11. — Diagrams of possible prophases of meiosis, involving three pairs of chromosomes; 1, barred, light, dark; 2, dots, rings; 3, stippled, white. *I. Leptotene.* Chromosomes in form of thread-like chromonemata. *II. Synaptene.* Homologous chromonemata fusing. *IIa. Synizesis*, another form of synapsis. *III. Pachytene.* Chromonemata fused, each one starting to duplicate itself, and also starting to exchange parts in 2 and 3. Only "pre-reduction" situation shown in this stage (see below). *IV, IVa. Diplotene*, shown enlarged in 1, 1a, etc. Members of pairs starting to separate, each chromonema now definitely duplicated to form a chromatid of a tetrad. In the barred pair pre-reduction is shown in *IV* and 1, post-reduction in *IVa* and 1a. In the other two pairs exchanges have occurred between the members of the pairs as indicated. Hence though the arrangement of parts varies, as shown in 2, 2a and 3, 3a, each separation in these cases is partly reductional and partly equational. In 4 and 4a two exchanges between a single pair of chromonemata is shown, a case not represented above or in Fig. 12. There are other possibilities. *V, Va. Diakinesis*, show possibilities of this stage following *IV* and *IVa*, respectively.

presumably separate the sister chromatids produced by duplication, and hence like any ordinary mitosis is *equational*. This order of events is called *pre-reduction*. If the sequence is reversed, it is *post-reduction* (Fig. 13). Actually, since all four chromatids of a group usually look alike, and since the number of remaining chromatids is the same in either case, there is generally no way of telling which type of division has occurred except in a few peculiar situations such as illustrated in Figs. 20, 21, and 22. Here post-reduction, though probably the more unusual type, can clearly be seen to have taken place. Obviously, however, the final result following the second division will be the same in either case. Also, because of certain further events, the terms "pre-" and "post-reduction" often lose their significance. These events are as follows:

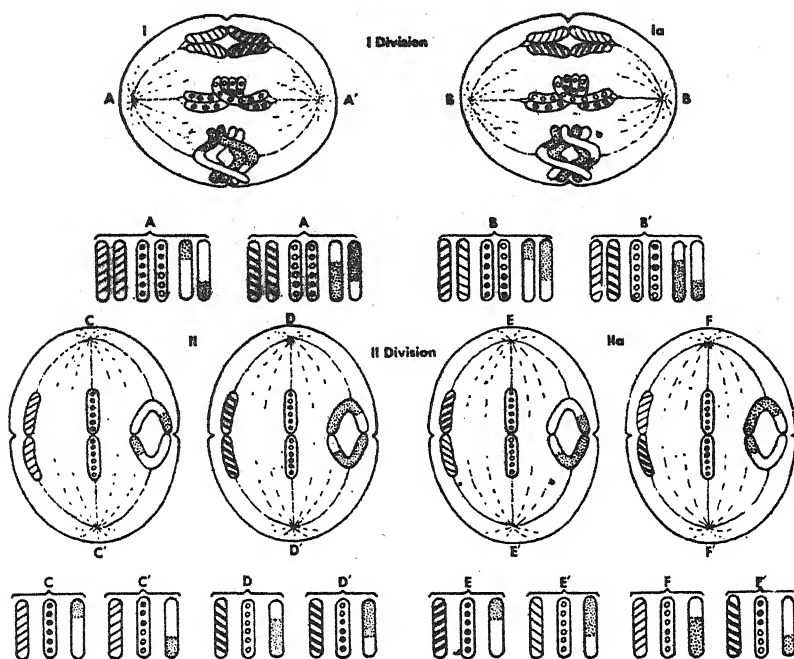


Fig. 12. — Continuation of diagram in Fig. 11, showing the *I* and *II* meiotic divisions. In *I* and *II* the barred tetrad, as in Fig. 11, is undergoing *pre-reduction*. In *Ia* and *Ia* the same tetrad is undergoing *post-reduction*, i.e., the *II* division is reductional (see text and Fig. 13). As indicated under Fig. 11, for the other two tetrads each division is partly reductional and partly equational. The groups of chromosomes bracketed under a given letter (*A*, *A* etc.) are those to be found in each cell following the division immediately above. Each tetrad behaves independently of the others, e.g., in *I*, cell *A* happens to receive the lightly barred pair of chromatids (chromosomes), but this is a matter of chance, and is unrelated to which pairs from the other sets of tetrads go to this cell. This is called independent assortment, and applies similarly to the single chromosomes of the *II* division. Hence many more combinations are possible than are shown above.

At some point after the quadripartite condition has developed, apparently in the pachytene or early diplotene, it is believed that exchanges of parts (genetic cross-overs, see below) frequently occur between the homologous chromonemata (chromatids) of a tetrad. While such exchanges may occur between one pair of homologues at one or more places simultaneously, and possibly between one pair at one place and the other pair elsewhere simultaneously, exchanges between members of both pairs seem never to occur simultaneously at the same place (Fig. 11, [2, 3, 4]). It should now also be noted that following such exchanges the initiation of repulsion between corresponding parts

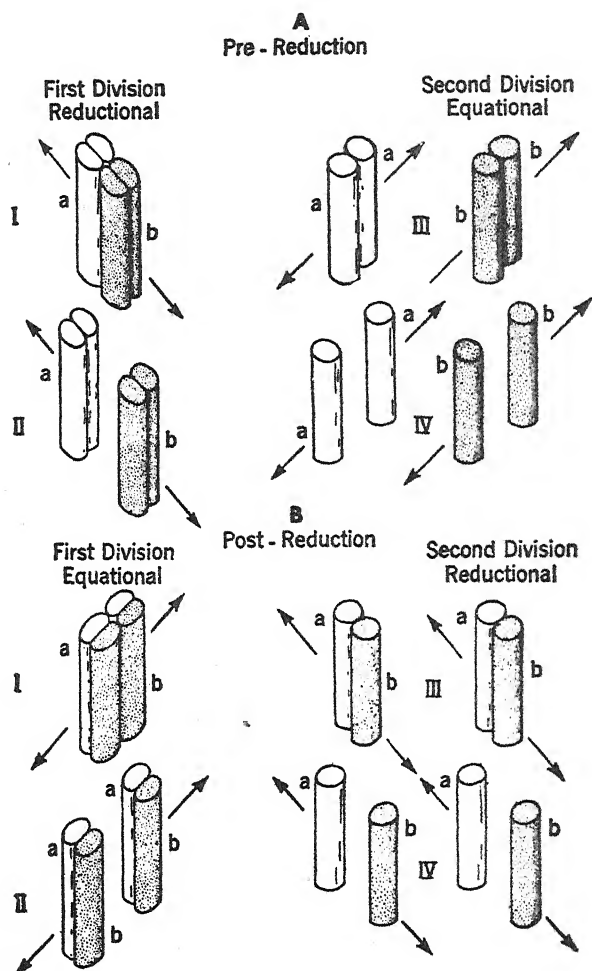


Fig. 13. — A stereoscopic diagram representing the two possible types of behavior of one of the three pairs of chromosomes indicated in Fig. 12 during the first and second meiotic divisions. The letter *a* designates one member of the pair and *b* the other member. For the sake of clearness, the plane of the second division is indicated in both types before the first division has actually started, in this manner producing a tetrad consisting of four chromatids. These chromatids are often definitely separate at this stage, or even as early as the diplotene stage (see text and Figs. 11, 12, 14).

In the upper set of four figures the first division (that on the left side) is reductional, i.e., *a* and *b* are separated from one another, while the second division (that on the right side) is equational, i.e., *a* and *b* are each split in half (*Pre-reduction*). In the lower set, on the other hand, the first division (that on the left side) is equational, i.e., *a* and *b* are each split in half, but in each instance the half of *a* remains attached to the half of *b*. The second division (that on the right side) then follows and in each half which resulted from the first division the *a* portion is separated from the *b* portion (*Post-reduction*).

of chromatids leads to a crossing of the chromatids as in Fig. 11. In the case of "pre-reduction," the repulsion will be between the corresponding parts of the homologues which attracted one another during synapsis, while in "post-reduction" it will be between corresponding parts of

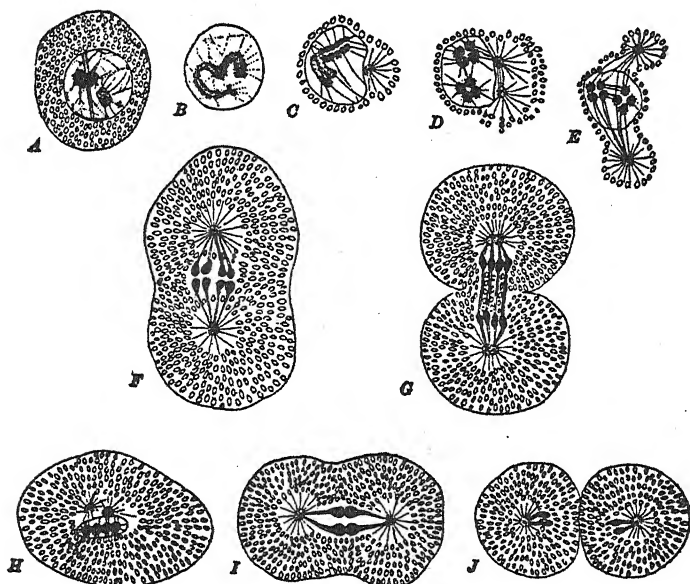


Fig. 14. — Tetrads formation in the spermatogenesis of *Ascaris megalocephala bivalens*. From Kellicott (*General Embryology*). After Brauer. $\times 795$. A-C. Stages in the division of the primary spermatocyte. A, B. Splitting, and C, condensation of chromatin thread, seen in side view. D. shows, in end view, that the splitting is double. Centrosome divided. E. Migration of centrosomes and formation of spindle. F, G. Division of the cell body and of the two tetrads. H. Secondary spermatocyte containing two dyads. I. Division of secondary spermatocyte. J. Two of the spermatids, each with two "monads" or single, univalent, chromosomes.

sister chromatids. In either case, crossing results, and the point of crossing is called a *chiasma* (plural *chiasmata*), the general situation being termed *chiasmotypy*.

In some forms the diplotene is followed by a so-called *confused* or *diffuse* condition in which the threads become less distinct, and approach the state seen in a "resting" nucleus. Either with or without the interpolation of this diffuse condition, there may also ensue a second contraction stage in which the threads are again drawn into a clump quite similar in appearance to that of the original synizesis in those cases where the latter occurs.

V. The Diakinesis Stage. — In this stage the chromatid threads, as in the case of any chromonemata approaching the metaphase stage of a cell division, undergo great shortening and condensation of chromatin. In the case of meiosis, however, the forming chromosomes differ from those of a similar mitotic stage in that they assume peculiar shapes, e.g., crosses, rings, etc. (Fig. 9 [10]; 11, V; 15, D, E), and are hence said to be *heterotypic*. This is due partly to the quadripartite nature of the chromatid groups, and partly to the twisting of the chromonema indicated above. The number of tetrad groups is of course haploid.

VI. The First Meiotic Division. — The above chromatids are presently arranged at the equator of an ordinary amphaster, but, because of the quadripartite character of the groups and the chiasmata involved, the metaphase figures, like those of diakinesis, have a peculiar appearance and are also termed heterotypic (Figs. 12, I, Ia; 15, A, B). As has been stated this division occurs along the more prominent of the diplotene separations, and in the case of a tetrad where no exchanges of chromonemal sections have occurred, the division will be exclusively reductional or equational, depending upon whether the separation is between homologous or sister chromatids. Even so, since all four chromatids of a tetrad look alike, there is usually nothing to show which type of division has occurred. Also where exchanges have taken place between homologues, each division is inevitably partly reductional and partly equational. In any event the resultant number of double chromatids, like the number of tetrads, will be haploid.

VII. The Second Meiotic Division. — Until the completion of the first division, the spermatocyte is known as *primary*. After that it is called *secondary*. The secondary spermatocyte generally enters upon a brief period of rest preceding the next division (Fig. 12, II, IIa). During this time the nucleus is often reconstituted, and the chromatin assumes to varying degrees the typical resting condition. Presently, however, the haploid number of double chromatids emerges from this stage in the usual manner, and becomes arranged on the spindle preparatory to the second division. Upon this occasion they generally present a normal appearance, aside from the important fact that their number remains haploid, and hence this division is termed *homotypical*.

From preceding discussion and reference to Figs. 11 and 12 it should now be clear why the question of pre- and post-reduction, as stated, often loses its meaning. Thus it may even be that the situation is different for different tetrads in the same nucleus. The only cases where pre- or post-reduction applies to the entire nucleus would be in organisms like the male of *Drosophila* where, for some unknown reason, there are no ex-

changes between any of the chromonemata. In instances where there are exchanges, however, reference to Figs. 11 and 12 makes it evident that in these cases two meiotic divisions are needed to effect complete separation of all homologous parts. Thus, considering parts 2, 2a and 3, 3a in the above figures, it is evident that each division as diagramed is, as noted, partly reductional and partly equational, and this is probably the

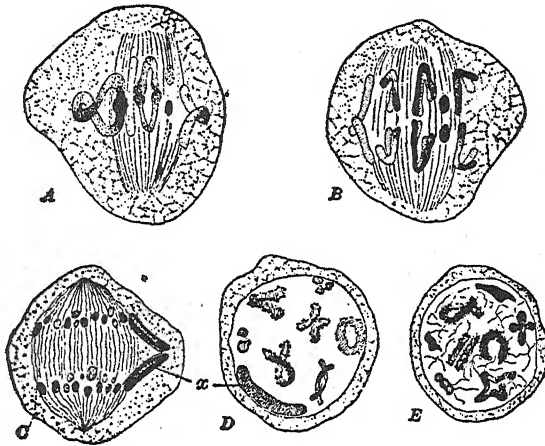


Fig. 15. — Meiotic divisions in certain Insects, showing forms of chromosomes and their relation to tetrads. From Kellicott (*General Embryology*). After de Sinety. x. 1125. A, B. Two stages in anaphase of primary spermatocyte division in *Stenobothrus parallelus*. Rings opening into Vs which diverge. C. Anaphase of spermatogonial division in *Orphanina denticauda*, showing differentiated chromosome, x. D, E. Preparation for first spermatocyte division in *Orphanina*, showing "tetrads" in various stages of formation from rings and crosses, i.e., diakinesis figures.

situation in the majority of cases, not only with respect to particular pairs of chromosomes, but with respect to all the pairs in a nucleus.

The above situation might be cited as a reason why two meiotic divisions are necessary, but this is not so. It is rather the duplication of chromonemata, probably in the pachytene previous to the exchanges of parts, which requires a subsequent second division in order to secure distribution of all homologous sections to separate nuclei. It is, therefore, the original duplication which needs explaining, and it appears that this phenomenon is simply inherent in all prophases. Hence a second division is inevitable whether needed to effect complete reduction or not. In any event, regardless of when reduction occurs, it is now evident that the

final result is the same; that is, there are produced four spermatids, each containing one haploid set of chromosomes with unique parts.

This last statement, it should be added, is frequently not precisely true. The exception is exceedingly important, but it has been omitted for the time being for the sake of clearness. It can be better appreciated, furthermore, when described in connection with the condition in the ovum. We shall reserve this point, therefore, until after the description of meiosis in the female.

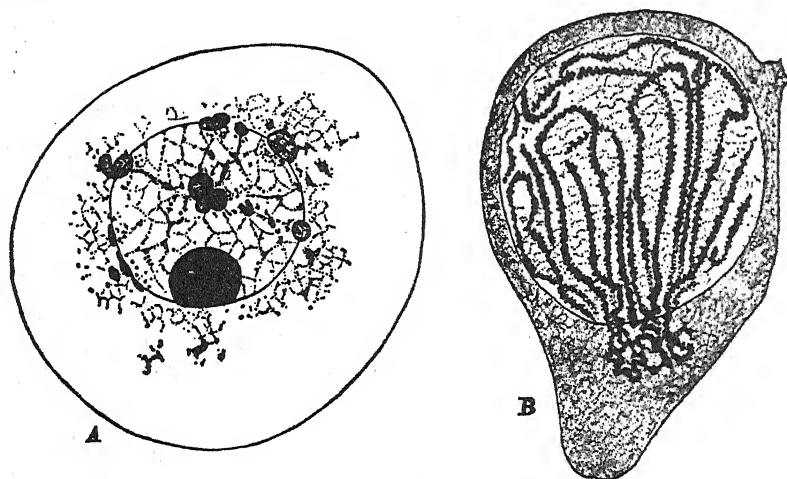


Fig. 16.—From Kellicott (*General Embryology*). A. Chromatin extrusion from the nucleus into the cytoplasm in the oöcyte of the Medusa, *Pelagia noctiluca*. After Schaxel. B. Extrusion of chromatin into the cytoplasm during the maturation of the oöcyte of *Proteus anguineus*. After Jörgensen. x 1080.

Meiosis in the Ovum.—Meiosis in the ovum is fundamentally similar to that in the sperm, with certain variations in detail. It will be possible, therefore, to make clear the process in the oöcyte by simply indicating the points in which it differs from that just described. These points may be stated as follows:

I. Length of Early Stages.—In some instances at least, the early meiotic stages up to and including synizesis occur immediately after the last oögonial division. As previously noted, however, these divisions are said in some cases to cease at the time of the hatching or birth of the female containing the cells in question. As indicated this is now denied with respect to Mammals, and is in doubt as regards all Vertebrates. In so far as it may occur, however, there follows the fact that certain of the meiotic stages must, in the cases of the last ova to mature,

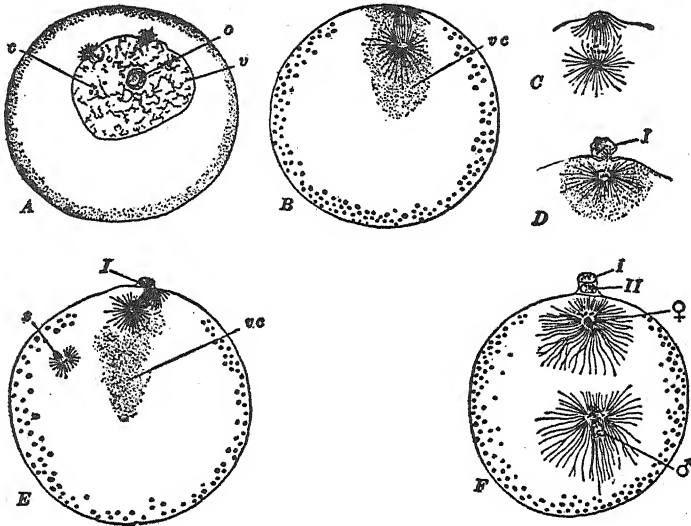


Fig. 17.—Meiosis and fertilization in the Nemertean, *Cerebratulus*. From Kellicott (*General Embryology*). After Coe. C, D, x 375, others x 250. A. Primary oöcyte. Part of the chromatin has been condensed into chromosomes, only five of which are shown (the number present is sixteen). The remainder of the chromatin is thrown out into the cytoplasm. The centrosomes, each with a small aster, are diverging, and the nuclear membrane is commencing to disappear. B. First polar spindle fully formed and rotated into radial position. Chromosomes in equatorial plate. The extra chromatin (vc) is seen scattering through the cytoplasm. C. First oöcyte division; anaphase. D. First polar body nearly separated. E. First polar body completely cut off; second polar spindle formed and rotating into radial position. Spermatozoön within the egg. F. Second polar body completely separated. Egg pronucleus forming, surrounded by large aster. Sperm pronucleus, also with a large aster, enlarged and approaching the egg pronucleus. These steps connected with the behavior of the egg and sperm nuclei (pronuclei) will be fully explained later on in the text.

c. Chromosomes. o. Nucleolus, vacuolated and commencing to disappear. s. Spermatozoön just within the egg. v. Germinal vesicle. vc. Extra chromosomal chromatin being scattered through the cytoplasm. I, II, First and second polar bodies. ♂ Sperm nucleus (pronucleus). ♀ Egg nucleus (pronucleus).

occupy very considerable periods of time. This is apparently not true of these stages in any of the sperm.

II. Loss of Chromatin.—In the oöcyte, a loss of chromatin into the cytoplasm has been alleged in a few special cases during the growth period, particularly in the diplotene stage (Fig. 16, B). That this phenomenon actually involves a loss of parts of the diplotene threads, however, seems unlikely for these threads or chromonemata presumably carry the genes, and any indiscriminate discarding of genes at any time

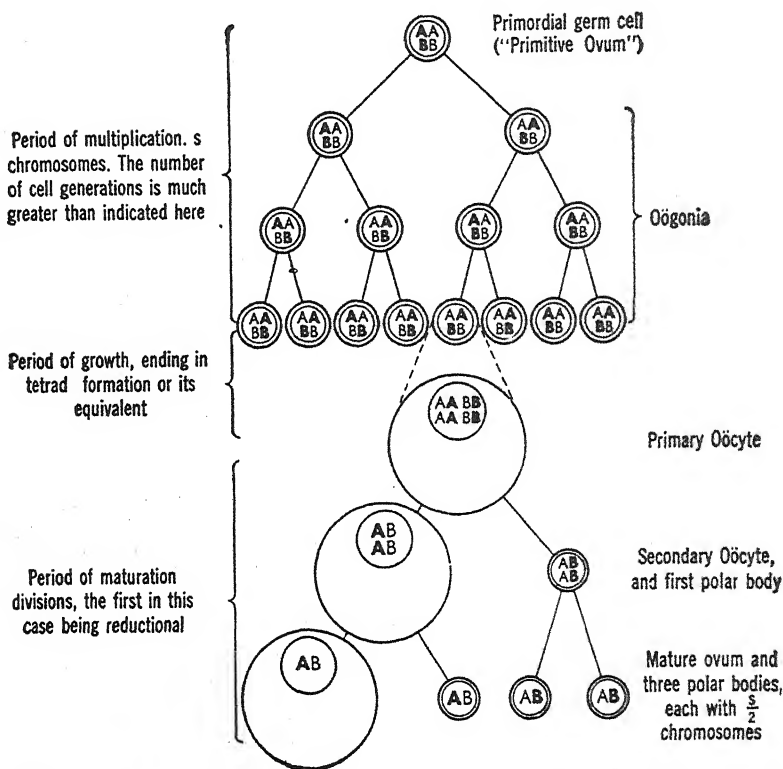


Fig. 18. — Diagram of the chief events of oogenesis. Modified from Kellicott after Boveri. The chromosomes are assumed to consist of two pairs represented by letters. AA represents one pair and BB the other. It is to be noted that the members of a chromosomal pair are not always dissimilar as the light and dark letters in this case suggest. They are so represented here in order to make apparent the distinction between the equational and reductional divisions during meiosis. Also as indicated in connection with Fig. 12, the dissimilarity of the members of one pair has no necessary relation to the dissimilarity of those of the other. Finally, it should be remembered that when dissimilarity between members of a pair of chromosomes does exist, it can rarely be detected by observation of the bodies themselves, only by the effects they produce.

is highly improbable. It, therefore, seems more reasonable that whatever loss there is in these cases concerns only the matrix material surrounding the coiled chromonemata, storage karyosomes, or the like (Figs. 16, A; 17). Such losses as these are not observed in spermatocytes.

III. Size of the Division Products. — Perhaps the most striking of all the differences between meiosis in the ovum and that in the sperm is the difference in the size and fate of the products of the two divisions. In the sperm, as, has been noted, the two meiotic divisions are equal

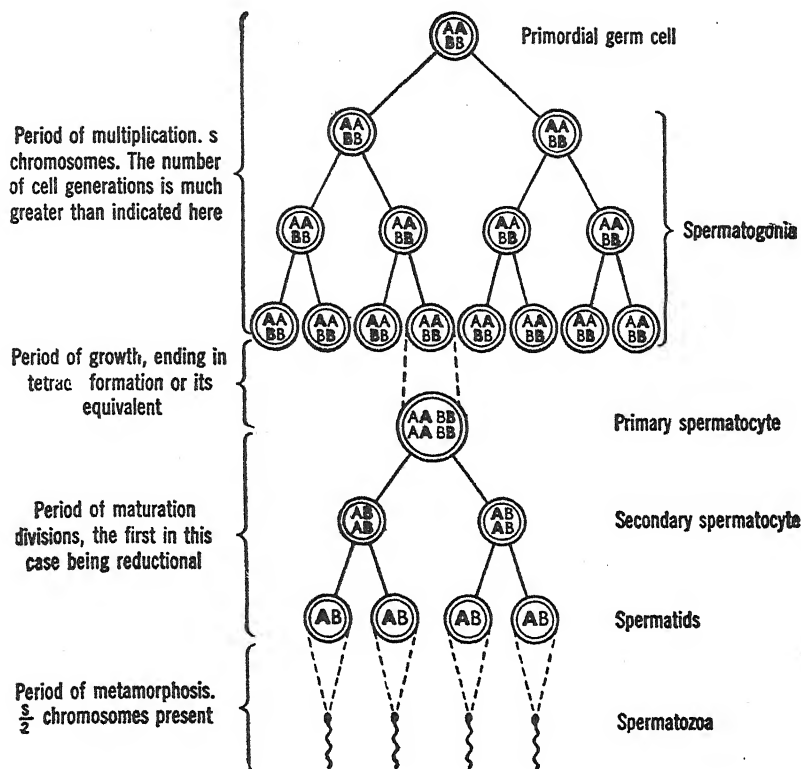


Fig. 19.—Diagram of the chief events of spermatogenesis. Modified from Kellcott after Boveri. The chromosomes are represented in the same manner as in the case of the ovum in Fig. 18. It will be noted that the light and dark members of the pairs are differently arranged relative to one another in the primordial and subsequent cells. This was done to indicate that this phase of the arrangement is purely a matter of chance. It might be the same in the case of the ovum or as suggested in that case the A and B might both be light or both dark in all the cells. Likewise starting with the combination shown in the primary oöcyte or the primary spermatocyte the four final cells in either instance might have had AB in two and AB in the other two instead of the combinations indicated. All that is required is that there be one member of each pair in each mature cell or polar body.

and the resulting four cells are all alike and functional. In the ovum, on the other hand, the cytoplasmic divisions in both cases are extremely unequal and only one of the four final products is a functional egg cell. The others are relatively minute and are known as *polar bodies*, the one resulting from the first division being termed the first polar body and that resulting from the second division the second polar body. This condition of inequality is brought about by the fact that at each divi-

sion the nucleus and division mechanism take up a position at the periphery of the cell instead of at its center. Thus one set of chromosomes remains in the main cell, while the other set is pinched off in a very small bit of cytoplasm (Fig. 17).

Although there is this great discrepancy in the distribution of the cytoplasm, there is good reason to believe that the nuclear content is the same in every case, just as it is in the sperm. In other words, the

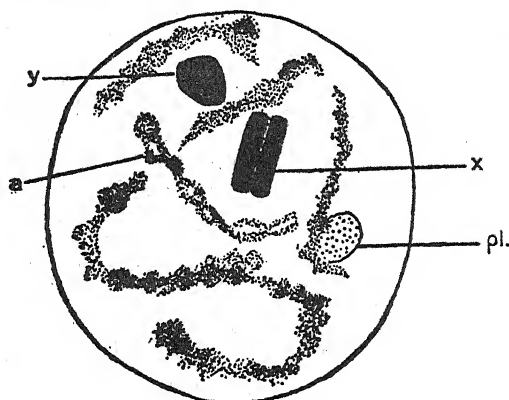


Fig. 20. — A diplotene nucleus in *Lygaeus bicrucis*. After E. B. Wilson. Note the condensed condition of the sex-chromosomes, *X* and *Y*. The remaining chromosomes, on the other hand, are still threadlike, one of them, *a*, as well as the sex-chromosome *X*, showing the characteristic diplotene split. This split in the case of the *X* is obviously equational. The plasmosome, *pl.*, is only partly visible.

performance is in every way homologous with the two spermatocyte divisions except for the inequality in the distribution of the cytoplasm. This idea is borne out by the fact that in many cases, as might be expected, the first polar body divides again as does its larger sister cell, thus producing one ovum and three polar bodies. This behavior in the case of the ovum is thought to be an adaptation to secure the

greatest amount of cytoplasm and nutriment in a single cell.

IV. The Time of the Meiotic Divisions. — In the sperm, as has been seen, meiosis is entirely completed within the testis and before the spermatid even enters upon its final period of development. In the ovum, on the contrary, meiosis is the last thing to occur. Sometimes division takes place while the ovum is in the ovary. More frequently, however, especially among the Vertebrates, at least one of the two divisions occurs after the ovum has left the gonad. Indeed in many cases the second division does not take place until after the egg has been entered by a spermatozoön (Fig. 17). A comparison of the chief processes involved in the development of the sperm and ovum is presented diagrammatically in Figures 18 and 19.

The Sex-Chromosomes. — We are now prepared to return to a consideration of the exception in chromosomal behavior which was

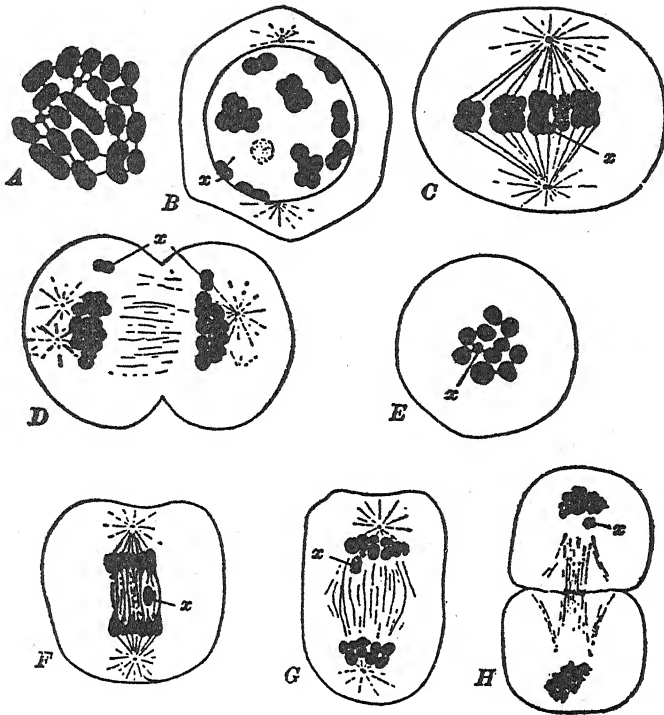


Fig. 21.—Meiosis during the spermatogenesis of the squash-bug, *Anasa tristis*, showing the behavior of the X-chromosome or idiochromosome. From Kellicott (*General Embryology*). A, After Wilson, others after Paulmier. A. Spermatogonium. Polar view of equatorial plate showing twenty-one chromosomes (ten pairs, plus one). The X-chromosome is not distinguishable at this time. B. Primary spermatocyte. Tetrads formed. C. Equatorial plate of first spermatocyte division. X-chromosome divided. D. Anaphase of same division. The daughter X-chromosomes have also diverged. E. Equatorial plate of second spermatocyte division. F. Anaphase of same division. The X-chromosome lies, undivided, between the two groups of daughter chromosomes. G. Late anaphase of same division. The undivided X-chromosome has passed to the upper pole, lagging behind the others. H. Telophase of same division. X-chromosome still distinct.

noted but not described at the end of the account of meiosis in the sperm.

In the somatic and germ cells of many animals, both male and female, there are found one or more chromosomes which in many cases behave quite differently from their fellows. They often stain more deeply, and are especially peculiar in that they frequently remain in the condensed condition during the entire growth period of the germ cells.

On this account they sometimes appear at this time like nucleoli with various distinctive shapes (Fig. 20). Also, during the anaphase stage of cell division, they are noted for a tendency to lag behind on the spindle (Fig. 21). One of the most striking things about these chromo-

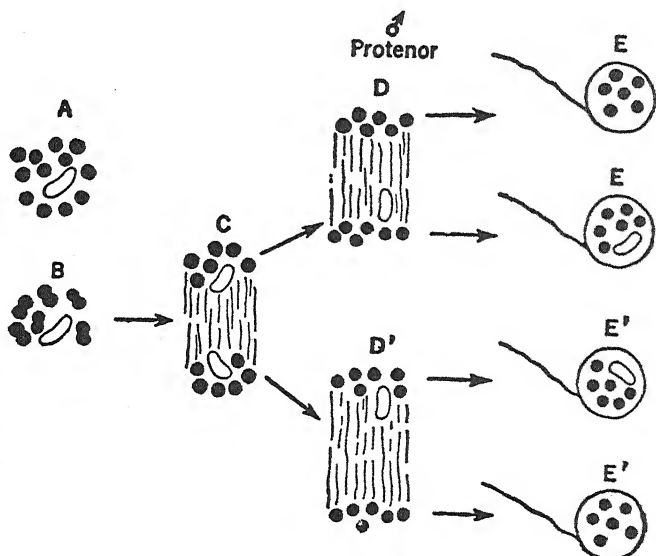


Fig. 22. — A diagram of the behavior of the chromosomes during the meiotic divisions in the male of *Protenor belfragei*. From Morgan (*Heredity and Sex*, published and copyrighted by the Columbia University Press). The sex-chromosome throughout is represented in outline, the others in solid black. *A*. The chromosomes in the somatic cell of a male. *B*. The chromosomes united in synapsis prior to the first meiotic division of a germ cell. The single sex-chromosome is without a mate. *C*. The first meiotic division, which for the sex-chromosome is certainly equational. *D*. The second meiotic division, "reductional" for the sex-chromosome, i.e., the latter goes to one pole or the other. It is impossible to say, certainly in this case, which division is really reductional for the ordinary chromosomes (autosomes). *E*, *E'*. The distribution of the chromosomes in the four spermatids resulting from the two meiotic divisions.

somes, however, is the fact that in some animals in the male, each somatic cell, as well as each unmaturation germ cell, possesses only one of them, while each cell of a similar type in the female has two. Under such conditions the one or two eccentrically behaving chromosomes are termed *X-chromosomes*. In such cases it follows of course that in the male the total number of chromosomes in each cell of the types indicated is odd, whereas in the female the number in each cell of a similar type is even.

Thus in the male of the insect *Protenor* the somatic cells and the un-maturated germ cells each possess 13 chromosomes, while similar cells in the female have 14 (Figs. 22 and 23). Under such circumstances it is obvious that when the male germ cell undergoes meiosis, its X-chro-

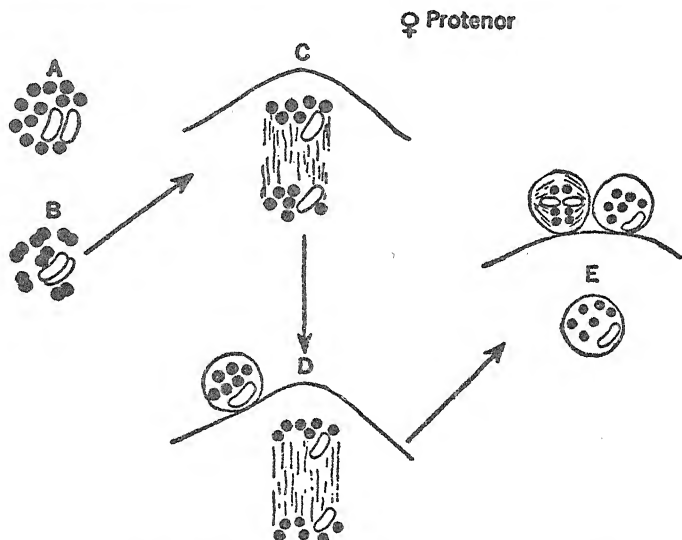


Fig. 23. — A diagram of the behavior of the chromosomes during the meiotic divisions in the female of *Protenor belfragei*. From Morgan (*Heredity and Sex*, published and copyrighted by the Columbia University Press). The sex-chromosomes throughout are represented in outline, the others in solid black. A. The chromosomes in a somatic cell of the female. B. The chromosomes united in synapsis prior to the first meiotic division of a germ cell. Note that in this case the sex-chromosome has a mate. C. The first meiotic division, probably equational, at least for the sex-chromosomes. D. The second meiotic division, which, if the first division was equational, is presumably reductional. E. The distribution of the chromosomes in the two polar bodies and the egg. The first polar body is represented as just undergoing the second division.

mosome will be without a mate. Apparently as a result of this fact the odd chromosome in the male only divides at one of the meiotic divisions, e.g., in the instance in question the first; and since this chromosome has not had a mate, its division must presumably be equational (Fig. 22, C). Following the second division, the final result, as usual, is four male germ cells, but their content is obviously not quite equal. Two of them possess six ordinary chromosomes (*autosomes*), while each of the other two possesses a similar six autosomes, and in addition an X chromosome, i.e., a total of seven (Fig. 22, D, E, D', E').

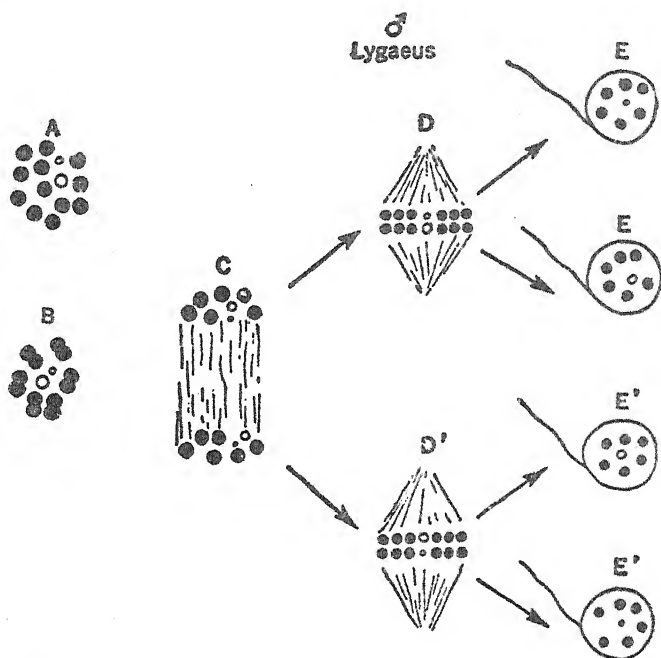


Fig. 24. — A diagram of the behavior of the chromosomes during the meiotic divisions in the male of *Lygaeus bicrucis*. From Morgan (*Heredity and Sex*, published and copyrighted by the Columbia University Press). *A*. The chromosomes in the somatic cell of a male. Note the large X and the small Y sex-chromosomes. *B*. The chromosomes united in synapsis prior to the first meiotic division of a germ cell. The X and Y do not usually unite at this time so that it is not indicated in the diagram (see Fig. 26). *C*. The first meiotic division in this case, so far as the sex-chromosomes go, is evidently equational. *D*. The second meiotic division, which for the sex-chromosomes is evidently reductional. *E*, *E'*. The distribution of the chromosomes in the four spermatids resulting from the two meiotic divisions, two receiving an X-chromosome and two a Y.

In the female since there are two X-chromosomes in the germ cells previous to meiosis each egg after meiosis will contain an X. This will also be true of course of the three polar bodies, but these being non-functional may be disregarded. Obviously, then, whether a fertilized egg is to contain one X or two will depend upon whether it is united with an X-bearing sperm or with one without an X.

There are numerous variations of this basic situation, the most common one being the type illustrated by the insect *Lygaeus*. Here the X-chromosome in the male does have a mate called the Y-chromosome, but it is different from the X, in this instance smaller, and can thus be distinguished from it (Figs. 24, 25, 26). A similar situation as regards

an X and Y pair of chromosomes occurs in Man. A slight variant of this arrangement is seen in *Drosophila*, the fruit fly, where the mate of the X in the male differs from it in shape rather than size (Fig. 27). There are still other situations where the X and Y are quite similar in appearance to each other, and even to the autosomes, but can be distin-

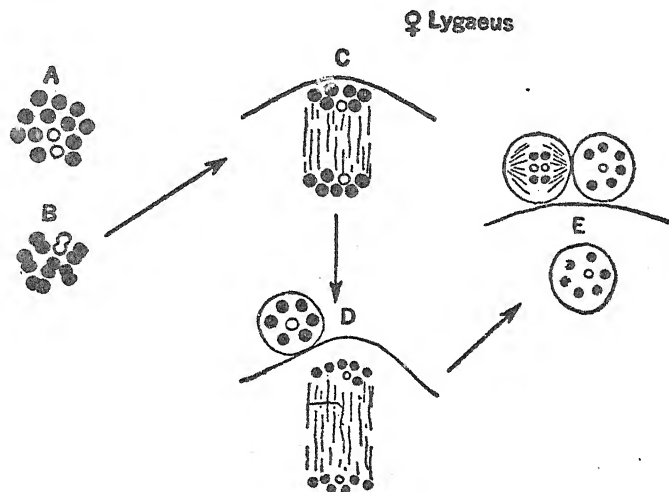


Fig. 25. — A diagram of the behavior of the sex-chromosomes in the female of *Lygaeus bicrucis*. From Morgan (*Heredity and Sex*, published and copyrighted by the Columbia University Press). A. The chromosomes in the somatic cell of a female. Note the two X-chromosomes. B. The chromosomes united in synapsis prior to the first meiotic division of a germ cell. C. The first meiotic division, probably equational. D. The second meiotic division, probably reductional. E. The distribution of the chromosomes in the two polar bodies and the egg. The first polar body is just undergoing the second division.

guished from the latter by their behavior, as already noted. In this last case, where the X and Y are not visibly distinguishable from one another, there is of course no obvious difference between the chromosomal condition in the male cell with its X and Y and in the female cell with a double X. There is good evidence from other sources, however, that even here fundamental qualitative differences do exist between the presumed X and Y chromosomes.

A more fundamental and striking variation in the relationships of these chromosomes occurs in Moths, Birds, and some Fishes. Here it is the female which has the odd chromosome, while the male has two of a kind. Since this arrangement was first observed in the moth *Abraxas* it is known as the *Abraxas* type. Also to avoid confusion the peculiarly behaving chromosomes are here termed Z and W instead of X and Y.

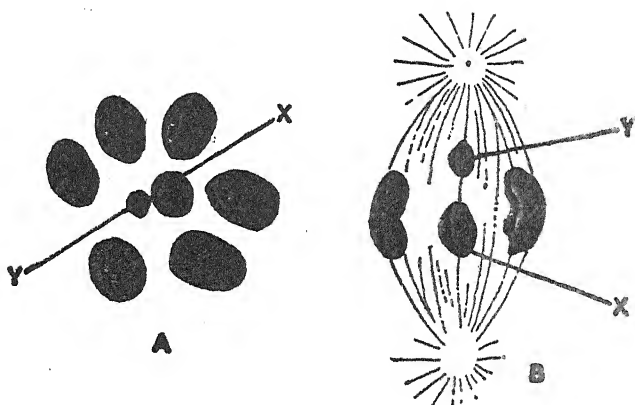


Fig. 26. — Division figures from the meiosis of the germ cells in the male of *Lygaeus bicrucis*. After E. B. Wilson. *A*. A polar view of the first meiotic division. In this insect the synapsis of the X- and Y-chromosomes not only does not occur while they are in a threadlike condition, but is postponed until almost the end of the first meiotic division. Even then it is evidently very slight, as indicated by the figure. *B*. A side view of the second meiotic division in the same animal. The chromosomes in this case do not lose their identity during interkinesis (i.e., the interval between the two divisions), and it therefore is possible to determine that the X and Y which united in synapsis at the end of the first division, as shown in *A*, are now being separated from one another. Thus for these chromosomes in this instance the second division is clearly reductional.

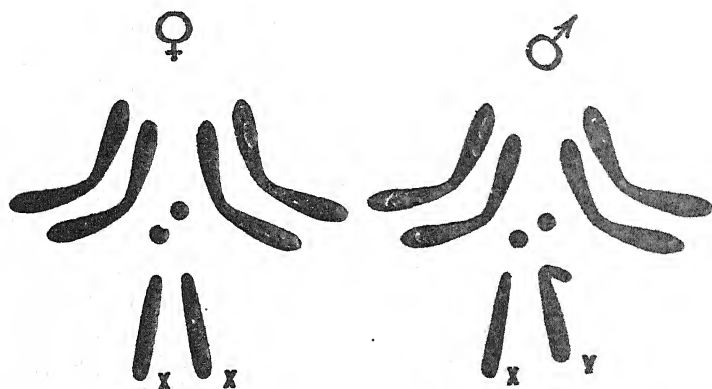


Fig. 27. — From the *Mechanism of Mendelian Heredity*, after Bridges. The female and male groups of chromosomes in *Drosophila melanogaster*, showing the four pairs of autosome chromosomes plus the XX pair in the female and the XY pair in the male. In this animal the members of each pair are usually found together as indicated.

It is then the ZZ combination which is found in the males and ZW in the females. Because of the evident relationship to sex which these XY or ZW chromosomes have, they are also termed *sex-chromosomes*. Something more concerning this important relationship will be said in a subsequent paragraph, but first a word is required regarding the entire meiotic phenomenon as so far described.

THE SIGNIFICANCE OF MEIOSIS

It is assumed that the student is aware of the evidences from his study of heredity and cytology that chromosomes are qualitatively different from one another with respect to chemical entities called genes or determiners. These genes, as is well known, are distributed from one end of a chromosome to the other, and with the exception of the sex chromosome in the male they normally occur in pairs. One member of a pair of genes is in one member of a pair of chromosomes, and the mate or allelomorph of that gene is in a corresponding position on the other chromosome of that pair. Thus it happens that at the reductional meiotic division one complete haploid set of chromosomes, and hence of genes, goes into one cell and another set into the other. The only normal exception to this is the case of the sex chromosome in the XY male, and the genes it carries. They go to one cell only. The non-reductional meiotic division is then similar to ordinary mitosis, and merely doubles the number of cells containing haploid sets. Fertilization of course involves the fusion of two germ cells, an egg and a sperm, and obviously the reduction of the chromosomes and genes at meiosis prevents them from being progressively multiplied at successive fertilizations. How this ingenious state of affairs came about is not known, and the speculations concerning it would take us too far afield in this text.

A further very significant parallel between the behavior of the genes and that of the chromosomes is as follows: It will be recalled that in the discussion of the heterotypic chromosomal figures of late diplotene and diakinesis it was suggested that part of the explanation for such figures was the fact that an exchange of sections had occurred between the homologous chromonemata (chromatids) of tetrads during the pachytene or early diplotene. It now remains to add that genetic evidence indicates that exchanges of blocks of genes, technically termed *cross-overs*, take place somewhere during the interval when the pachytene and diplotene stages are visible. The exact time is uncertain, but that these gene exchanges are in some way definitely related to the exchanges between homologous chromonemata is generally admitted as beyond doubt.

SEX DETERMINATION

It is known that the XY chromosomes, more particularly the X, also carry genes, and some of them apparently concern sex. Genetic evidence

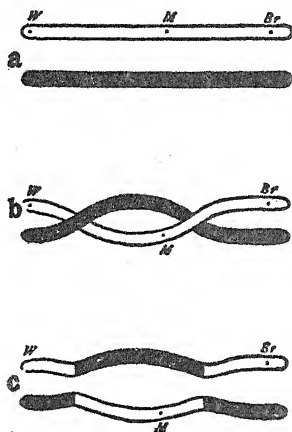


Fig. 28. — Diagram to illustrate crossing over. From Morgan (*Mechanism of Mendelian Heredity*). The white and the black rods (a) twist and cross at two points (b). Where they cross they are represented as uniting (shown in c). That an interchange of pieces has taken place in the region between genes *W* and *Br* is demonstrated from the standpoint of inheritance by breeding experiments. The results of these are most readily explainable on the assumption that the gene *M* has gone over to the other chromosome.

from normal and abnormal cases indicates that the sex genes in the X-chromosome tend to produce female characteristics, or at least to produce the initial impulse in that direction, while those in the autosomes tend to produce male characters. This of course applies only to the non-Abraxis type of sex inheritance where the male contains the single X. The situation is evidently reversed in the other types. Thus the determination of sex is a matter of balance between two types of gene influence. Normally a diploid set of autosomes balanced against a single X produces a male, while the addition of another X is enough to produce a female. All genes, however, produce their effects by interacting not only with each other, but with their surroundings. Thus we know that various inherited tendencies can be modified by the proper environment. So it should not be surprising that sex also is subject to environmental influences. In some animals, like Birds and Mammals, it is rather hard to alter the surroundings of the developing organism very much. In other animals like Amphibians, however, this is easily possible, and in such crea-

tures various environmental changes have been tried. It has thus been found that proper temperatures at critical periods (Witschi, '29, see Chap. VI), and other appropriate procedures are able to reverse the sex which a particular chromosome complex would have produced in a more normal situation. Also changes in the internal environment, such as endocrine secretions, might be expected to alter the development of sex characters, and experimental evidence shows that this is true, not only in Amphibians, but in both Birds and Mammals.

F

FERTILIZATION AND EARLY STAGES IN DEVELOPMENT

FERTILIZATION

BEFORE proceeding to an account of development in any particular animal, it may be well to discuss certain processes which are always involved, and to note the chief methods of their occurrence.

Fertilization in all higher forms consists of the union of an egg and a spermatozoön. This union may occur within some cavity of the female into which the sperm have been introduced, or it may occur outside. The latter is the more common method among animals which live in the water. In either case, thousands of the relatively minute sperm are required to insure the fertilization of each single egg by one spermatozoön. We shall now turn to a generalized account of the process.

PENETRATION

The Action of the Sperm. — Both eggs and sperm contain certain substances similar to hormones, hence called *gamones*. Those in the sperm are *androgamones*, one which prevents premature excessive sperm activity thus conserving their energy, and another which dissolves the gelatinous membrane surrounding many eggs. Those in the egg, on the other hand, are *gynogamones*, one of which, at an appropriate time, counteracts the first androgamone, thus increasing sperm activity, and a second which makes the sperm heads sticky, causing them to adhere to the egg surface. In addition, some eggs may secrete something which attracts sperm. Penetration of the egg may take place at any point of the surface, or the sperm may enter through a special orifice, the *micropyle*. Usually only one sperm enters (*monospermy*), and in case more do so development is generally abnormal. Sometimes, however, in relatively large yolked eggs, several sperm normally enter, a phenomenon called *polyspermy*. Even in such cases only one of the spermatozoa takes active part in the further events of fertilization. The remainder eventually degenerate and disappear; previous to this they may divide several times, and perhaps aid in breaking up the yolk to make it more assimilable.

40. FERTILIZATION, EARLY DEVELOPMENT

In such cases they are referred to as *merocytes*. The method by which the extra sperm are excluded in the event of monospermy will be discussed presently.

As soon as the head of the sperm has punctured the surface of the egg, the swimming movements of its tail cease. In some cases the latter is regularly drawn into the egg along with the head and middle piece, while in others it is left outside. In either event it soon degenerates and takes no more part in the fertilization process.

The Reaction of the Egg.

The Perivitelline Space and the Fertilization Membrane. — Probably the first and most characteristic reaction of almost all eggs to puncture by a sperm is the formation of a space between the egg surface and its innermost covering (i.e., in most instances the vitelline membrane). It is called the *perivitelline space* and seems in some cases to be due to the pushing away of the membrane by a secretion from the egg. In other instances it may be due to shrinkage of the egg or to absorption of water by some substance between the membrane and the egg surface. In any event such a separation of the egg from its covering of course makes the latter more conspicuous, and even in such eggs as have seemed previously to lack a membrane, one now becomes visible. Because of this increased visibility following fertilization, the membrane about the perivitelline space, whether it be the original vitelline membrane, one apparently newly formed, or a fusion of both of these, is frequently called henceforth the *fertilization membrane* (Fig. 46, D). The significance of the phenomenon just noted is not well understood. It was thought at one time to aid in preventing polyspermy. Since eggs from which the membranes have all been entirely removed continue to be impervious to further fertilization, however, it is evident that this condition is not the result of the existence or the location of any membrane. It has also been maintained that the obvious alteration in position of the membrane is accompanied by increase in its permeability to gases and other substances. That there is considerable basis for this belief is indicated by the fact that in some instances there is a decided increase in oxidative processes and other phenomena requiring such a change.

The Changes in the Egg Cytoplasm. — Aside from these phenomena connected with the inner egg membrane, fertilization also initiates certain other changes in the egg proper. Almost simultaneous with the appearance of the perivitelline space there is frequently evident an out-

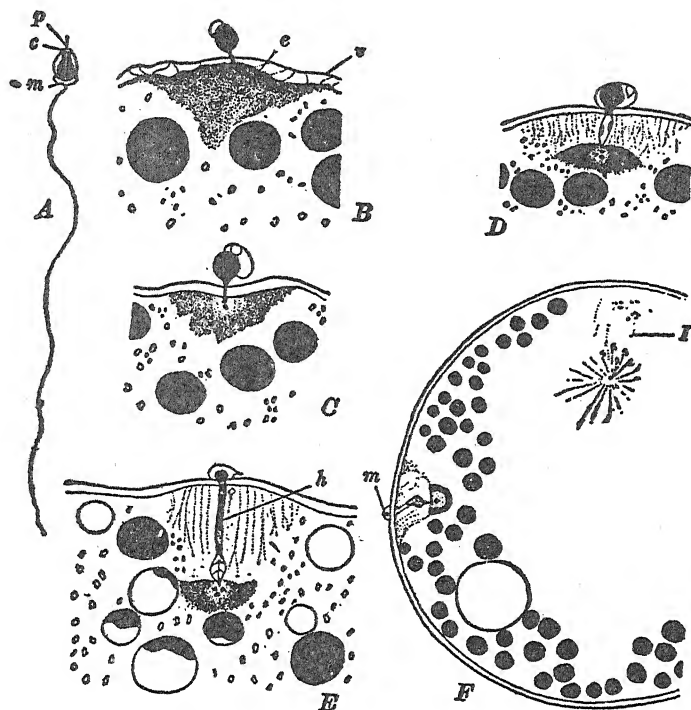


Fig. 29. — Entrance of the spermatozoon in the fertilization of the Annu-
 lulate, *Nereis limbata*. From Kellicott (*General Embryology*). After
 Lillie. A. Spermatozoon. B. Perforatorium has penetrated egg membrane;
 entrance cone well developed. Fifteen minutes after insemination. C.
 Thirty-seven minutes after insemination. D. Entrance cone sinking in
 and drawing the head of the spermatozoon after it. Forty-eight and one-
 half minutes after insemination. E. Head drawn in still further. Forty-
 eight and one-half minutes after insemination. F. Entrance completed.
 First meiotic division in anaphase. Fifty-four minutes after insemination.
 The middle piece, as well as the tail, remains outside.

c. Head cap. e. Entrance cone. h. Head of spermatozoon (nucleus). m.
 Middle piece. p. Perforatorium. v. Vitelline membrane. I. First polar divi-
 sion figure.

pushing of the cytoplasm at the point where a spermatozoon has pene-
 trated the fertilization membrane. This protuberance is then entered
 by the sperm, and because of this fact it is often termed the *entrance*
cone (Fig. 29, B). Following these events both the cone and the parts
 of the sperm which it contains are apparently drawn down into the
 deeper egg substance (Fig. 29, C, D, E). Besides this somewhat local-
 ized activity on the part of the cytoplasm, however, there are also evi-
 dences of other effects which seem to be more widespread. Thus, since

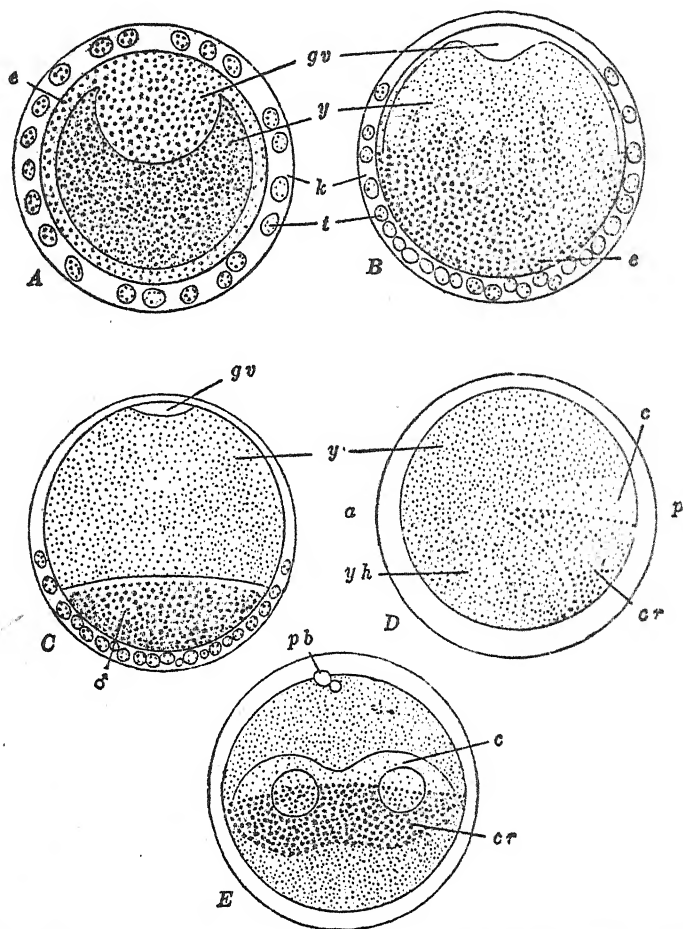
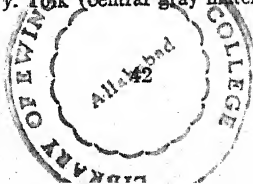


Fig. 30. — Total views of the egg of Tunicate *Cynthia partita*, showing changes in arrangement of materials of egg subsequent to fertilization. From Kellicott (*General Embryology*). After Conklin. x 200. A. Unfertilized egg, before fading out of germinal vesicle. Centrally is gray yolk; peripherally is protoplasmic layer with yellow pigment, and surrounding egg, the test cells and chorion. B. About five minutes after fertilization, showing streaming of superficial layer of protoplasm toward lower pole where spermatozoön enters, and consequent exposure of gray yolk of upper hemisphere. The test cells are also carried toward lower pole. C. Side view of eggs showing yellow protoplasm at lower pole; at upper pole a small clear region where polar bodies are forming. The location of sperm pronucleus (nucleus) is also indicated. D. Side view of egg shortly before first cleavage, showing posterior collection of pigmented protoplasm (yellow crescent) and clearer area above it. E. Posterior view of egg during first cleavage, showing its relation to the symmetry of egg.

a. Anterior. c. Clear protoplasm. cr. Yellow crescent. e. Exoplasm or cortical layer, with yellow pigment. g.v. Germinal vesicle. k. Chorion. p. Posterior. p.b. Polar bodies. t. Test cells. y. Yolk (central gray material). y.h. Yellow hemisphere. σ Sperm nucleus.



polyspermy is not prevented by the fertilization membrane, it is held that such prevention may be due to a general alteration in the egg cytoplasm.

More specifically, according to one theory the entrance of the sperm is made possible by the interaction of a substance in or on its head with another substance on or near the surface of the egg. This latter substance is called *fertilizin*, and such part of it as is not used up in the interaction with the sperm is supposed to be immediately eliminated by interaction with another substance called *antifertilizin*. This latter material is thought to be located more deeply within the egg cytoplasm, and is brought into contact with the fertilizin by a rearrangement of the egg materials produced by the entrance of the sperm. All the fertilizin having thus been eliminated, no further fertilization is possible (Lillie, '19). Though this explanation of events is still theoretical there is considerable experimental evidence for it in certain organisms. Also, whether or not this be true, evidence is not wanting that in some cases at least, all of the egg cytoplasm is profoundly disturbed by the sperm entrance. It seems likely indeed that this is more or less true of all eggs, but the disturbance is particularly obvious in certain instances because in these instances different regions of the egg cytoplasm are differently colored and thus distinguishable. In such eggs it has therefore been possible to observe that, following fertilization, a sudden and marked rearrangement of these parts of the cytoplasm takes place. Such, for example, is the case with the egg of the Tunicate, *Cynthia (Styela) partita* (Fig. 30), and also with that of *Amphioxus* (see below).

THE LATER STAGES

The later steps in the fertilization process which are now to be described are all more or less directly connected with the fusion of the nuclei of the sperm and egg.

The Egg Nucleus.—The meiotic divisions of the egg are sometimes entirely completed previous to fertilization. More usually, however, as in the case of most Vertebrates, only one of these divisions occurs before the sperm entrance, and in some instances (e.g., *Nereis*) both are delayed until after this event (Fig. 32, *B, C*). In these cases where meiosis has not begun, or is unfinished prior to the penetration of the sperm, the latter event seems to act as a stimulus which causes the meiosis to proceed. As soon as it is completed the egg nucleus is definitely formed, and the centrosome which took part in the second division disappears.

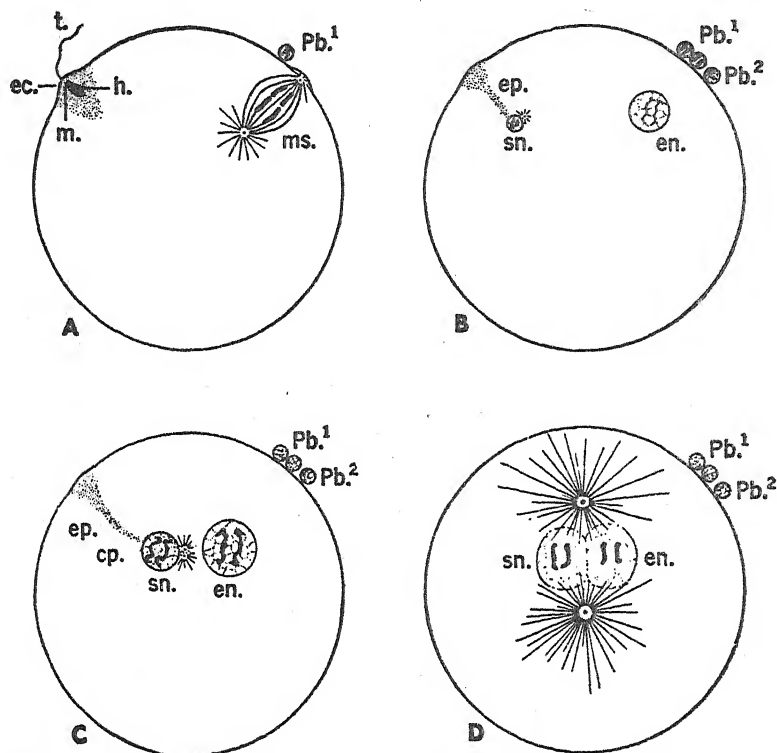


Fig. 31.—A generalized diagram of the penetration of the sperm and the fusion of the egg and sperm nuclei, the haploid number of chromosomes being assumed in this case to be two. The trail of pigment marking the path of the sperm actually occurs only in the case of the Frog's egg. The egg membranes are not represented. Compare the stages with those in Fig. 32 showing corresponding processes in the egg of *Nereis*.

A. The first polar body has been given off, and the second meiotic division is in progress. The sperm head and middle piece have entered the egg, leaving the tail outside. B. The first polar body has divided and the second has been given off, while the completed egg nucleus has started to move toward the center of the ovum. The sperm nucleus consisting of the sperm head has enlarged somewhat, has partially rotated, and is also moving toward the center of the egg. The new division-center has appeared in the region of the middle piece. C. The two nuclei are enlarging and approaching one another. The sperm nucleus, having completed its rotation, has altered the direction of its movement somewhat (not always necessary), to hasten their meeting, and the division-center is dividing into two parts. D. The nuclei, each containing the haploid number of chromosomes, have started to fuse. The division-centers, each consisting of a centriole and centrosome and surrounded by its aster, have taken up their places preparatory to the first division of the egg.

cp. Copulation path. ec. Entrance cone. en. Egg nucleus. ep. Entrance path. h. Head of sperm. m. Middle piece of sperm. ms. Meiotic spindle of the second meiotic division. pb¹, pb². First and second polar bodies. sn. Sperm nucleus. t. Tail of sperm.

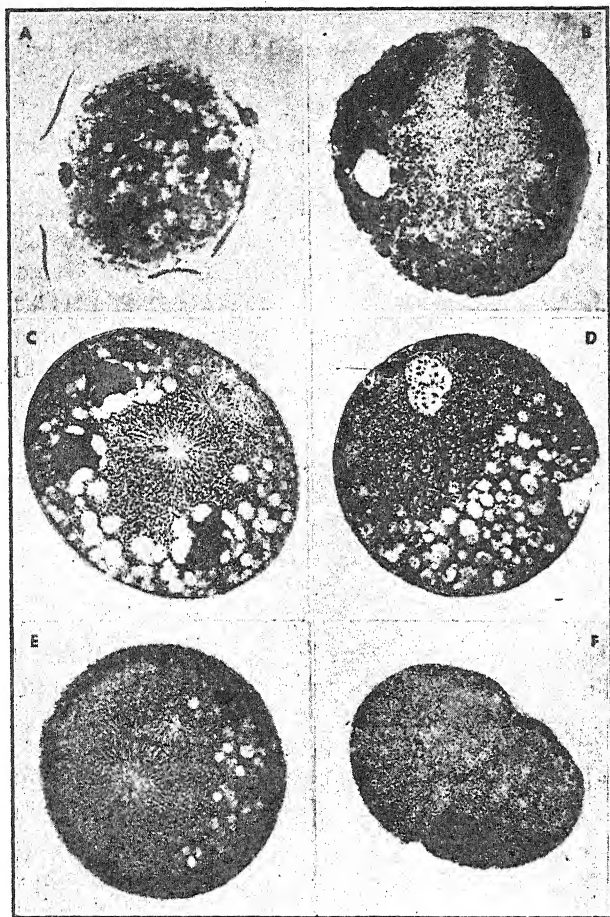


Fig. 32. — Photomicrographs of sections of *Nereis* eggs, showing stages in fertilization meiosis and cleavage. The photographs were made in the Anatomical Department of Western Reserve University Medical School from specimens presented to that department by Professor O. Van der Stricht, and are reproduced by the courtesy of Professor Van der Stricht and Dr. E. W. Todd.

A. At the top of the figure the spermatozoon is shown just entering the egg. The egg membrane is broken, and separated from the egg at various points. B. The first meiotic division spindle. C. The first meiotic division has been completed, and the first polar body lies outside the egg beneath the egg membrane. It appears at the top of the figure and slightly to the right. Just within the egg in the same vicinity is the second meiotic spindle, while at about the center of the egg is the sperm head with its aster in front of it. D. The egg and sperm nuclei in the upper left hand part of the egg are fusing, while just beneath the egg membrane is one of the polar bodies. E. The division spindle for the first cleavage. F. The first cleavage is completed and parts of the asters for the second cleavage are indistinctly visible in the two daughter cells.

The Sperm Nucleus and the Division-Center. — While this completion of the meiotic divisions is taking place, the head and the middle piece of the sperm advance into the egg.¹ Also, as this occurs these parts rotate through an angle of 180° so that the middle piece is in the lead (Fig. 31, *A, B*). The advance then continues along a course whose first portion is called the *entrance* or *penetration path*, and which, in the case of the Frog, is marked by granules of pigment. Meanwhile the acrosome which effected the entrance of the sperm has disappeared, while marked changes are also taking place in the nuclear portion of the head and the middle piece. The former is enlarging, and within it the chromatin is forming a typical nuclear reticulum. In the region of the middle piece, on the other hand, a centriole and centrosome appear and are presently surrounded by a small aster. It has been claimed that this centriole is identical in whole or in part with the centriole (or one of the centrioles) which entered the middle piece during the transformation of the spermatid. This is very doubtful, and in many cases is certainly not true. It does seem, however, that in most instances the new division-center at least arises under the influence of the middle piece.

The Fusion of the Egg and Sperm Nuclei. — Previous to or during the above processes, the second meiotic division of the egg has been concluded, and the egg nucleus has moved from the periphery of the cell into approximately the midst of the active cytoplasm (Fig. 31, *D*; Fig. 32, *D*). Of course in telolecithal eggs with a large yolk, this point will be just below the surface of the animal pole, rather than at the actual center of the egg. The new sperm division-center and nucleus, which have meanwhile been advancing along the penetration path, now move directly toward the egg nucleus. This in many instances may involve a slight change in the course of the sperm, and when such is the case the latter portion of its course is termed the *copulation path* as distinguished from the first portion or entrance path (Fig. 31, *C*).

As the nuclei meet each other their membranes disappear. Also there has appeared in each the haploid number of chromosomes² (Fig. 31, *C, D*). Meanwhile the sperm division-center and aster divide, if indeed they have not already done so, and form a typical division spindle. Upon this spindle the restored number of chromosomes arrange them-

¹ In some instances; e.g., Nereis, the middle piece, as well as the tail, remains outside.

² In many cases the chromosomes are not actually visible as such until after the fusion of the pronuclei. In these instances the number appearing in the single fusion nucleus is then diploid as would be expected.

selves, and each is then divided in the usual manner preparatory to the first cleavage of the egg (Fig. 32, *E*). It should be noted that in this process there is no fusion of the chromosomes. On the contrary, this event, presumably the actual climax of the entire phenomenon, does not occur until the period of synapsis in the germ cells in the new individual, as described above.

THE CONSEQUENCES OF FERTILIZATION AND THEIR IMPORTANCE

We may now consider briefly some of the apparent results of this process and their possible importance. There have been three main consequences of fertilization which have been held to be of vital significance, though as will appear, none of them has proved to be necessarily dependent on this phenomenon. They are as follows:

I. **Reproduction.** — It has been said that the chief result of fertilization is to bring about reproduction, (a) by restoring the diploid number of chromosomes, and (b) by furnishing or causing to develop a new kinetic division-center. This argument is unsatisfactory for the following reasons:

1. Granting that these events take place in connection with reproduction, the answer is, nevertheless, superficial. For the question immediately arises, why should the egg lose half its chromosomes and its division-center, thus making fertilization necessary before reproduction can occur?

2. There are numerous cases of both artificial and natural parthenogenesis, showing that neither the extra chromosomes nor the new division-center is absolutely necessary.

3. Finally the fact that the union of two cells so frequently precedes reproduction may be explained thus. Let us assume that there is some reason, such as those indicated below, why a mixture of different strains of protoplasm is beneficial. It then follows that in a Metazoan, the only time such a mixture can possibly occur is when the protoplasm of the animals concerned is in the form of single cells, i.e., the germ cells. Then since the animals are in fact Metazoa, the union of the germ cells must eventually be followed by cell division in order that the Metazoan condition may again be reached. Under such circumstances, the multiplication obviously is not proved the result of the fertilization.

II. **Rejuvenescence.** — It has been widely held that the fusion of different strains of protoplasm which occurs during fertilization is necessary to bring about a revivifying of any given race of animal or plant.

Without this, it is held, cell division will gradually become less frequent, and will finally cease. The chief argument for this view has been furnished by certain experiments on Protozoa. Thus, Calkins ('19) seemed to prove this by work with *Paramecium*, although earlier studies by Woodruff ('14) had appeared to show that some strains could be kept going indefinitely by an internal reorganization called *endomixis*. Later work by Jennings ('44), Sonneborn ('39), and others has shown the situation to be even more complicated than had first appeared. Thus, conjugation sometimes prolongs the life of certain lines, and sometimes not. At all events it is evident that the mixing of different strains of protoplasm is at least not universally necessary for revitalization.

III. Variation. — Fundamentally, of course, variation depends upon changes in the genes. As modern genetics has shown, however, the actual appearance of these variations in an animal or plant may sometimes depend upon the shuffling and recombinations of the genes which meiosis and fertilization bring about. Also in some instances significant variations may result from the abnormal behavior of whole chromosomes or sets of chromosomes, which in a few instances is definitely known to have produced new species. Weismann was entirely ignorant of the details of all these processes as now understood, but he did have some rather elaborate theories concerning normal meiosis and fertilization. He termed the recombinations of genetic determiners, which he correctly believed came about through these latter events, *amphimixis*, and he considered that variations so caused were an important source of material upon which natural selection might act. Others, e.g., Hertwig, believed that the shuffling and recombining processes tended to cancel out the effects of gene mutants and thus helped to keep the race constant. As a matter of fact it is now clear that both points of view are correct in different cases. It also appears that evolution could occur without the fertilization process, though probably not so rapidly.

Conclusion. — In view of the above facts, the general conclusion as to the function of fertilization may perhaps be stated thus: While it seems reasonable that the process is an important one in view of its wide occurrence, we do not as yet understand its full significance. It does appear likely, however, that recombinations of genes favorable to renewed vigor, and also to production of variations, are involved. Advantages of this nature, while not essential for life, may well have been great enough to have favored the evolution of sex and the correlated phenomenon of fertilization.

EARLY STAGES IN DEVELOPMENT

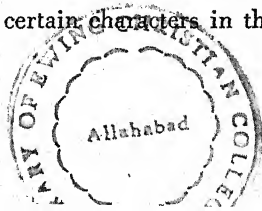
RELATIVE INFLUENCE OF EGG AND SPERM ON THESE STAGES

In the above discussion of the germ cells it has been stated that despite the great disparity in the cytoplasmic content of the ovum and sperm, their influence upon development is approximately equal. The abundant egg cytoplasm is simply for the purpose of supplying food and material for the nuclear factors to work upon, and varies according to requirements in these respects. The sperm cytoplasm, on the other hand, is only for the purpose of bringing its nucleus to that of the inert egg, and possibly of initiating division. Indeed the very features which characterize the cytoplasm of a particular egg or sperm are presumably determined by genes within the chromosomes, just as are the features which characterize the adult animal.

Nevertheless, it must now be noted that the character of the egg cytoplasm does determine in a rather obviously mechanical way, and apparently sometimes in more subtle ways, the nature of the early stages in development which we are about to consider. The cytoplasm of the sperm, however, though often strikingly variable in form, is apparently without any such influence. Because of this fact, in the case of most of the animals whose embryology is to be studied, it will be necessary to give a rather full account of the ovum and its development. The various kinds of spermatozoa, on the contrary, will need little further attention.

RELATION OF GENETICS AND EMBRYOLOGY

Before proceeding with a general description of the first steps in development, it is perhaps pertinent to say a few words at this point concerning the relationship between the field of genetics on the one hand and that of embryology on the other. This text deals primarily with the latter, yet the term gene or determiner has been frequently employed, and quite evidently these entities are supposed to be significant controlling elements in development. As a matter of fact the subject matter of these two disciplines, i.e., genetics and embryology, like that of physics and chemistry, is becoming constantly more interrelated. In the earlier days of these subjects the geneticists were more concerned with showing how genes were distributed during the reproductive process. They also sought to prove that their occurrence in certain combinations always resulted in the appearance of certain characters in the



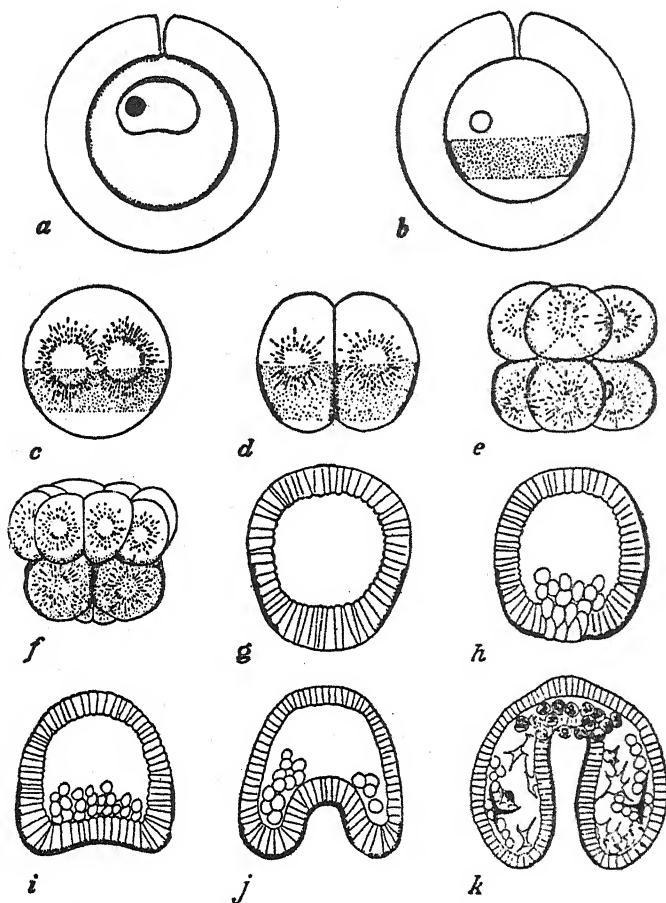


Fig. 33.—Cleavage in the Sea-urchin, *Strongylocentrotus lividus*. From Jenkinson, after Boveri. Animal pole uppermost in all cases.

a. Primary oocyte surrounded by jelly, and containing large germinal vesicle with nucleolus. Pigment uniformly distributed over surface. b. Ovum after formation of polar bodies. Pigment forms a band below the equator. c, d. First cleavage. e. Eight-cells. Pigment almost wholly in lower quartet (vegetative blastomeres). f. Sixteen-cells. The lower quartet has divided latitudinally and unequally, forming four micromeres at the vegetal pole; the upper quartet has divided meridionally forming a plate of eight cells. g. Section through blastula. h. Later blastula, showing formation of mesenchyme at lower pole. i, j, k. Three stages in gastrulation, showing the infolding of the pigmented cells to form the hypoblast (archenteron). In j the primary mesenchyme is separated into two masses, in each of which a spicule is formed (k). In k the secondary, or pigmented, mesenchyme is being budded off from the inner end of the archenteron.

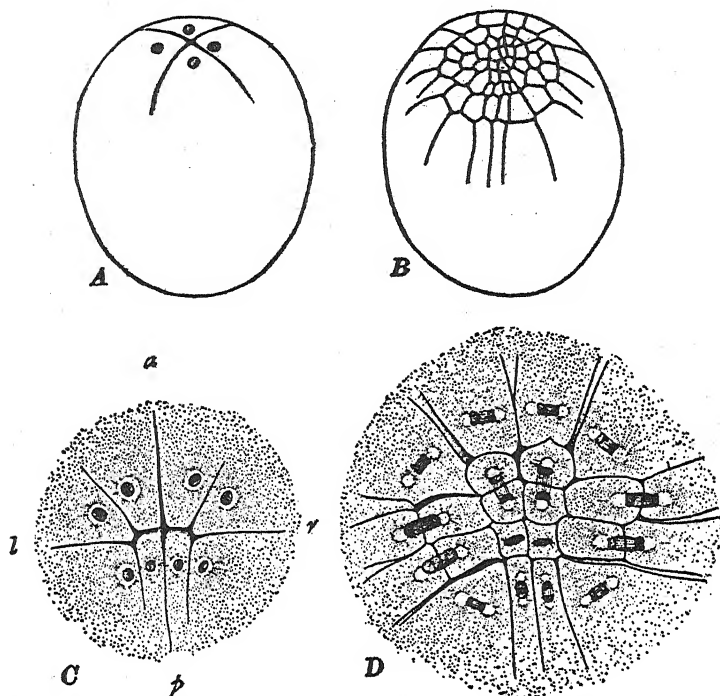


Fig. 34. — Meroblastic cleavage in the Squid, *Loligo pealii*. A, B. Egg viewed obliquely, showing animal pole. $\times 45$. From Kellicott (*General Embryology*). After Watasé. C, D. Surface views of animal pole, more highly magnified, to show bilateral arrangement of blastomeres. From Wilson, "Cell," after Watasé. A. Four-cell stage. B. About sixty cells. Cells at the animal pole very small, lowermost cells incomplete, cell walls extending down toward the uncleaved lower pole. C. Eight-cell stage. D. The fifth cleavage (sixteen to thirty-two cells).

a-p. Marks the plane of the first cleavage and the median plane of the organism. l-r. Marks the second cleavage, and the transverse plane of the organism.

adult animal or plant. The embryologists, on the other hand, were occupied mainly with describing the steps in development. Presently, however, both groups came to ask the question: How do the genes act to produce their end results? This has led to a rapid rapprochement between the students of the two fields. The geneticists have tried to find out how genes interact with each other and with their cytoplasmic environment to cause the development of the adult characters. Also, as already suggested, the embryologists on their side have ceased to be interested in merely describing what happens, and are now actively engaged in experiments to find out how it happens. Thus both groups are,

so to speak, approaching the same goal from opposite sides. When they meet, and we know how all the genes act to produce all the end results, the problems of embryology will be solved. Meantime, enough remains to be done from both directions to keep us all busy for a long time.

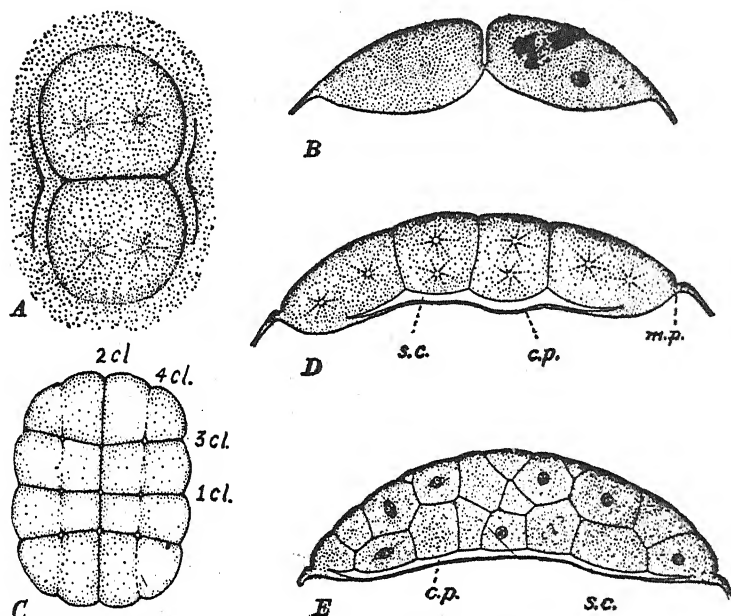


Fig. 35.—Cleavage in the Sea-bass, *Serranus atrarius*. From H. V. Wilson. A. Surface view of blastoderm in two-cell stage. B. Vertical section through four-cell stage. C. Surface view of blastoderm of sixteen cells. D. Vertical section through sixteen-cell stage. E. Vertical section through late cleavage stage.

c.p. Central periblast. m.p. Marginal periblast. s.c. Segmentation cavity (blastocoel).

SEGMENTATION

Subsequent to the first division of the egg which has been indicated, further divisions follow each other, often in relatively rapid succession. The period of these early divisions is termed that of *segmentation* or *cleavage*.

Types of Cleavage.—As has been suggested above, the type of cleavage is largely determined by the nature of the egg cytoplasm, particularly as regards the amount and distribution of the yolk which the latter contains. In a homolecithal egg with relatively little yolk, the cleavage is *total* or *holoblastic*, and approximately equal (Fig. 33)

The equality in the size of the cells decreases, however, as the amount of yolk increases. This follows from the fact that where there is much yolk present, it is never equally distributed. Instead it gathers on one side, i.e., the vegetal side, so that the ovum becomes telolecithal. Then since yolk-filled cytoplasm divides with more difficulty than cytoplasm that is free from yolk, inequality of division necessarily results. It is termed simply *unequal cleavage* (Fig. 61). Finally in cases where the amount and density of the yolk is very great, as in many Fishes and Birds, that part of the egg which contains it does not cleave at all, or only very slightly. In such eggs, as already noted, the yolk-free cytoplasm exists only as a small accumulation at the animal pole of the egg, called the *blastodisc*. It is then chiefly this disc which divides; after division it is called the *blastoderm*. Cleavage of this type is known as *meroblastic*, or *discoidal* (Figs. 34, 35).

The Blastula. — After cleavage has continued for a time in an egg of the homolecithal type a hollow sphere of cells results, with a cavity at or near its center (Figs. 33, *g*; 36, *A*). Such a sphere is called a *blastula*, and the cavity at its center is termed the *segmentation cavity*, or *blastocoel*. In eggs of the markedly telolecithal type there also exists at the completion of cleavage a sphere, but in this case, as has been noted, the greater part of it consists of undivided yolk. It is nevertheless termed a blastula, and the segmentation cavity will lie at the animal pole between the largely unsegmented yolk mass and the blastoderm (Figs. 35, *D, E*; 37, *A*). Although cell division continues the cleavage stage may be said to end when the blastula condition has been reached.

GASTRULATION

Gastrulation, as the name implies, has to do with the formation of the primordial gastric or gut cavity called the *archenteron*. In many cases this cavity is entirely separate from the blastocoel from the beginning of its formation, but in others complete separation comes later. In any event in addition to the formation of the gastrular cavity the process also usually involves the setting apart of two of the three primordial germ layers with which all higher animals start their differentiation. These first two layers are sometimes referred to as the *ectoderm* and *endoderm*, the former being on the outside and the latter lining the archenteron. This, however, is not quite correct because the third layer, called *mesoderm*, to be referred to presently, is necessarily derived from one or the other or both of the two already formed. Hence at least one of these is really more than ectoderm or endoderm for it contains the

elements of the mesoderm. Therefore the one from which the third is derived in cases where this origin is clear, is often temporarily termed *mesectoderm* or *mesentoderm* as the case may be. Another pair of terms frequently applied to these two layers are *epiblast* for the outer layer and *hypoblast* for the inner one. These terms are noncommittal so far as indicating which is to give rise to mesoderm, and it is therefore convenient to use them, up until the time that this last-named layer appears. After that each of these layers can be referred to by its definitive name, ectoderm, mesoderm, and endoderm. This is the procedure which will be followed in this text. It should be further added that in some Invertebrates, like the earthworm, the mesoderm actually arises before gastrulation by the budding off of cells into the blastocoel. After this budding off of the mesoderm, the remaining wall of the blastula might then be called ectoendoderm, since it is this wall which later becomes differentiated into definitive ectoderm and endoderm during gastrulation. Among Vertebrates, however, events appear to be always in the order indicated.

Gastrulation having been thus defined, it now becomes necessary to indicate briefly and in a general way the processes through which it may occur. For the sake of clearness and convenience these processes will be described separately, though it should be noted that in the majority of actual cases two and often more of them take place together.

Invagination. — Probably the simplest method of gastrulation is by *invagination*, a method which is sometimes spoken of as being typical. As a matter of fact, however, the accomplishment of gastrulation by this means alone is rather exceptional even among the Invertebrates, and among the Vertebrates it never occurs to the exclusion of other methods. Indeed within the latter phylum it is found in a relatively unmodified form only among a few of the very lowest members of the group. In all the higher animals it is very largely altered and augmented by other means, and in many instances appears not to be present at all. In its simplest and most unmodified condition, however, it may be described thus:

Let the blastula be thought of as a hollow sphere, one hemisphere of which is to be regarded as the animal half and the other hemisphere as the vegetal half, while the cavity within the sphere is the blastocoel (Fig. 33, g; 36, A). Now, imagine the vegetal half to be pushed in or invaginated until it almost touches the animal half opposite to it. The sphere has thus become a gastrula. The original blastocoel has been virtually obliterated and a new cavity has been formed by the invagina-

tion. This is the archenteron, and it is lined by the original vegetal cells which may now be termed hypoblast (Figs. 33, *k*; 36 *B*). The cells which constitute the animal hemisphere, on the other hand, are now called epiblast. The opening of the archenteric cavity to the exterior is then in this case the *blastopore*, and the rim of this opening the *lip of the blastopore*. It must be immediately stated, however, that only in eggs of a relatively yolkless character, is the blastopore thus a wide-open orifice. As the amount of yolk increases it tends to fill both the archen-

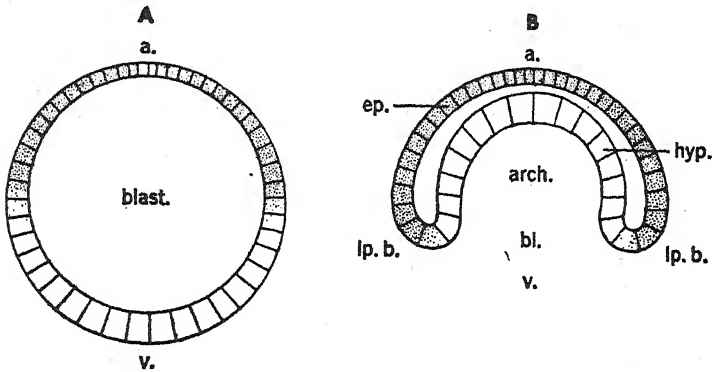


Fig. 36. — Diagrammatic representation of gastrulation by invagination. *A*. Ideal meridional section of a blastula. *B*. Ideal meridional section of a gastrula. *a.* Animal pole. *arch.* Archenteron. *blast.* Blastocoel. *bl.* Blastopore. *ep.* Epiblast. *hyp.* Hypoblast. *lp.b.* The lip of the blastopore or germ ring. *v.* Vegetal pole. The cells at the vegetal pole are usually larger because they contain more yolk.

teron and its opening more and more, until in eggs of the extremely telolecithal type there is very little left of the archenteron as a cavity or of the blastopore as an opening. Thus in eggs of this sort the boundary of the blastopore, i.e., the blastoporal lip, is really the edge of the blastoderm. To cover all cases, therefore, it is perhaps better to describe the lip of the blastopore as the line of undifferentiated tissue where epiblast and hypoblast merge with one another. This description it will be found applies to the edge of the blastoderm as well as to the rim of a blastopore which possesses a wide opening. It may now be added that the lip of the blastopore is also often called by another name, i.e., the *germ ring*. The reason for this is the fact that it was once thought that a very large portion of each side of the embryo always originated from this ring in a manner to be described below (see conrescence). A further word will be said on this topic when the latter process is discussed.

Involution.—A second process of gastrulation may be described as involution or inflection. It is very common among the Vertebrates, and, within this group at least, it probably always accompanies any invagination which may occur. In many cases also it appears to be the chief factor involved, particularly among forms arising from a telolecithal egg. Therefore we shall study involution in a telolecithal egg.

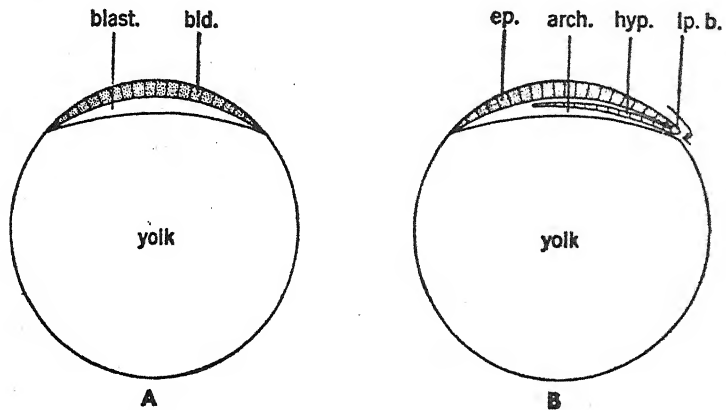


Fig. 37.—Diagrammatic representation of gastrulation by involution in the case of an egg with a large yolk mass which does not segment. *A.* Ideal meridional section of a blastula. *B.* Ideal meridional section of a partially completed gastrula, bisecting the dorsal blastoporal lip. *arch.* Archenteron. *blast.* Blastocoel. *bld.* Blastoderm. *ep.* Epiblast. *hyp.* Hypoblast. *lp.b.* The lip of the blastopore. The arrow points to the blastopore, and indicates the movement of involution.

In such eggs it has been noted that the yolk usually does not segment at all, and that in correlation with this the blastocoel will be greatly reduced (Fig. 37, *A*). Under such conditions it is evident that gastrulation cannot occur by simple invagination because the mass of yolk filling the center of the blastula will not permit it. What does happen, therefore, is this: At some point on the edge of the blastoderm (see above), the dividing cells, instead of extending out over the unsegmented yolk, begin to be turned over the blastodermal rim, i.e., *involved* into the segmentation cavity. These inturned cells then constitute the hypoblast, while those which remain without are epiblast (Fig. 37, *B*). According to definition, therefore, the edge of the rim, in this case the edge of the blastoderm, is the blastoporal lip or germ ring, while the movement over this lip is designated as *involution*. As suggested above, however, this process is not confined to animals with a large yolk mass, and it is to be clearly understood, therefore, that the

only essential feature concerned is the passage of cells over the lip. It is this movement, which, as stated, comprises involution, and this remains true whether the active cells be arranged in the form of a blastoderm or otherwise. In some instances where the yolk mass is very great, as in many Fishes, the movement is accompanied by no invagination. In others (Amphioxus and Amphibians), the latter process also takes place to a greater or less extent. In any event the inflection or involu-

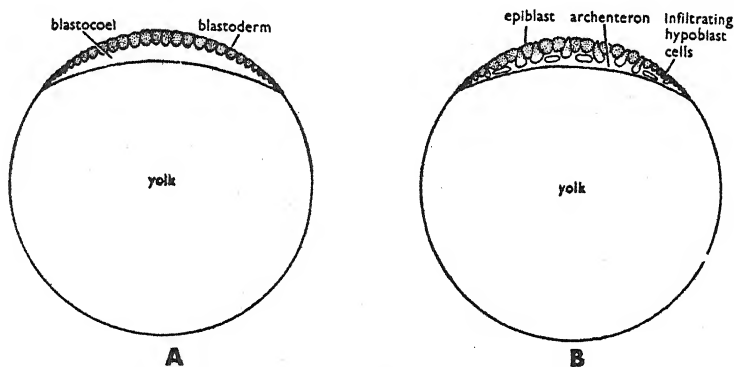


Fig. 38. — Diagrammatic representation of gastrulation by infiltration in the case of an egg with a large yolk mass which does not segment. *A.* Ideal meridional section of a blastula as in Fig. 37. *B.* Ideal meridional section of a partially completed gastrula, showing some of the cells of the blastoderm creeping inside the former blastocoel, and spreading out there to form the hypoblast.

tion is most active in that portion of the blastoporal lip which eventually proves to be dorsal. The degree and character of its occurrence in other parts of the lip vary considerably in different animals, and can best be indicated later in specific cases.

Mechanisms Concerned in Invagination and Involution. — Before proceeding to a description of the next methods of gastrulation, it seems well to pause here to consider the possible mechanisms involved in the processes already described. As has been indicated the essential feature in either invagination or involution is the movement of cells over or around an edge or lip. This type of movement, moreover, is an important aspect of various other cell rearrangements in embryology, as for example in the enterocoelic formation of mesoderm and the development of neural folds to be described later. Hence an effort to discover the mechanism involved here has been one of the important points of attack by the experimentalists. What makes a ball of cells invaginate? What makes cells roll over a margin? The answer seems to be that it is

due to a change in the shape of the cells as suggested long ago by Rhumbler, Butschli, T. H. Morgan and others. This can be easily understood if one imagines a hollow ball of cells such as depicted in Figure 36. If one notes especially the bigger cells in this figure it is clear that they are larger at their outer ends. It is also clear that so long as they retain this shape it will be very difficult or impossible for them to roll inward. If, however, the cells at one pole of the egg, or in the case of tel-

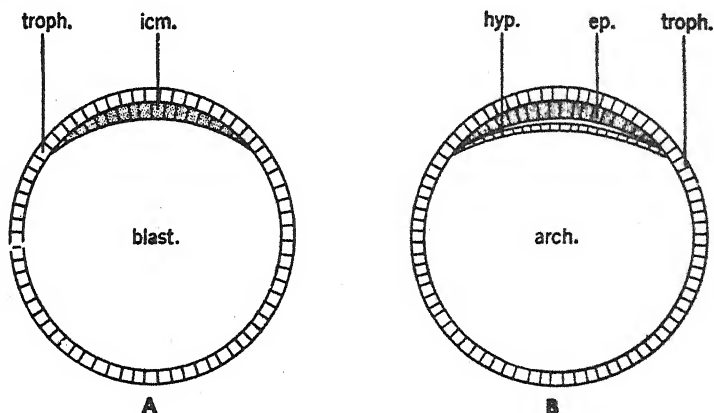


Fig. 39. — Diagrammatic representation of gastrulation by delamination. *A.* Ideal meridional section of a blastula, or as it is called in Mammals, the blastocyst. *B.* Ideal meridional section of a gastrula. *arch.* Archenteron, as yet only partially lined by endoderm and lacking a blastopore. *blast.* Blastocoel. *ep.* Epiblast. *hyp.* Hypoblast. *icm.* Inner cell mass, virtually homologous with the blastoderm of blastulas with much yolk. *troph.* Trophoblast, an embryonic layer peculiar to Mammals. (See chapter XIV.)

olecithal eggs, at the blastoporal lip, should become smaller at their outer ends, the case would be different. Then their tendency would be to behave just as they actually do behave in invagination or involution, i.e., to roll inward around the margin of a lip. That this is what occurs, seems now to be quite evident. The question remains, however, as to what makes cells in such situations change their shape. Here experiment is still seeking a complete answer. However, according to some investigators it is most probably due mainly to a higher alkalinity of the blastocoelic fluid. This in turn causes a change in surface tension in different regions of the cell membranes of the cells concerned (Holtfreter, '44, Lewis, '47). Thus if the tension at the inner ends of these cells became relatively less than at their outer ends, the inner ends would tend to become larger. One can and must of course then go still further back and ask why the tension changes in these cells and not in

others. This and related questions have not all been satisfactorily answered, but their asking points the way in which investigation must proceed.

Infiltration. — Heretofore this process has not been recognized as a method of gastrulation. Recent investigations on the Chick, however, seem to indicate that possibly such a term is appropriate to describe what takes place there and perhaps also in the Mammal. In any case it involves simply the inwandering or infiltration of cells from the blastoderm, or its homologue, into the space beneath (the blastocoel). This space may or may not be largely filled with yolk. In the Chick of course it is so filled, while in the Mammal it is not. In either event the cells thus originating soon spread out to form a continuous layer of hypoblast, and the former blastocoel becomes the archenteron. The infiltration process, if and where it occurs, is, like invagination and involution, probably due to the change in shape of some of the cells of the original layer. Each cell concerned, becoming larger at its inner end, tends to form, as it were, a sort of pseudopodium, and crawl into the blastocoel (Fig. 38).

Delamination. — A fourth process by which gastrulation may occur is that of delamination, and so far as Vertebrates are concerned it has been supposed to take place most typically in Mammals. However, just as infiltration may be involved to some extent in this group, so may delamination occur to a certain degree in the Birds. According to Brachet, delamination of a sort also plays a small part in a rather special manner in the gastrulation of the Amphibian. This will be considered more fully when we come to the Frog. At any rate the process, wherever it may occur, consists simply in the separation or splitting off of cells from a pre-existing layer or mass. These cells then become confluent, as in the case of those derived by infiltration, to form the hypoblast (Fig. 39).

It should be noted that where gastrulation occurs wholly, or almost wholly, by either infiltration or delamination, or both, no real blastopore exists, at least at first, and hence apparently there can be no blastoporal lips. It will be recalled, however, that the blastoporal lips have been defined in general as the region where the epiblast meets and merges with the hypoblast. Furthermore it may be stated that even in the cases of gastrulation almost or wholly by infiltration or delamination the epiblast and hypoblast do ultimately come into contact around the rim of the blastoderm, and also in another region to be noted later. Hence the essential part of the definition of a blastoporal lip still holds

for both the places referred to. This problem will be discussed at greater length when the cases of the Chick and the Mammal are reached.

Accessory Processes.—Two other processes are probably always to some extent involved in gastrulation, and in most instances are of considerable prominence. As will presently appear, however, these movements, at least among Chordates, are not strictly a part of gastrulation

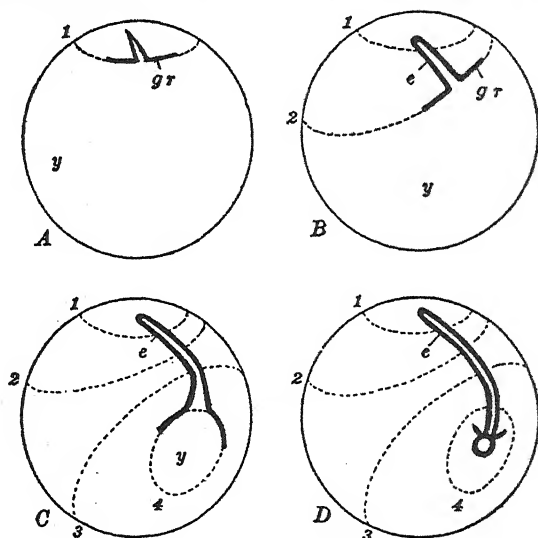


Fig. 40. — Diagrams illustrating four stages in the formation of the Teleost embryo (having an extremely telolecithal egg), and the growth of the germ ring or lip of the blastopore around the yolk mass (epiboly). From Kellicott (*General Embryology*). After O. Hertwig. *e*. Embryo. *gr*. Posterior margin of the germ ring. *y*. Yolk mass. 1, 2, 3, 4, successive positions occupied by the germ ring as it advances over the yolk.

proper; i.e., they do not actually differentiate hypoblast from epiblast, though they aid in the extension and disposition of both these layers. Hence they may be more correctly regarded as accompanying or accessory activities.

I. Epiboly.—This is the first of these accessory movements, and occurs most typically in the development of eggs possessing abundant yolk, e.g., those of Fishes and Amphibians. It merely involves the gradual growth of the blastoporal lip over the yolk, or the yolk-filled vegetal cells. It may be roughly pictured (Fig. 40) by imagining a solid sphere, the yolk, over which a rubber cap, the blastoderm, is being stretched, the rim of the cap representing of course the lip of the blasto-

pore. The movement, however, is not due apparently to any actual process of stretching, but rather to active cell division in the overgrowing layers, and this activity is thought to be most intense in the region of the lip itself, i.e., the germ ring. It may be also that in this case, too, the movement is augmented by surface-tension changes which produce a creeping of the cellular rim over the yolk. At all events the result of such a process will obviously be eventually to enclose the yolk as in a sac (the *yolk sac*); the completion of this process necessarily involves also the closure of the blastopore (Fig. 40).

II. Concrescence and Convergence. — The process of concrescence as contrasted with that of convergence is one whose occurrence, as previously suggested, is now seriously questioned. At least this is

true with the conception of it originally held. Nevertheless in order to understand what is now believed it seems best to indicate the essentials of the original theory. It may be described thus. As the process of epiboly goes forward there always results, as noted, a gradual drawing together of the blastoporal lips, so that the size of the blastopore itself is dimin-

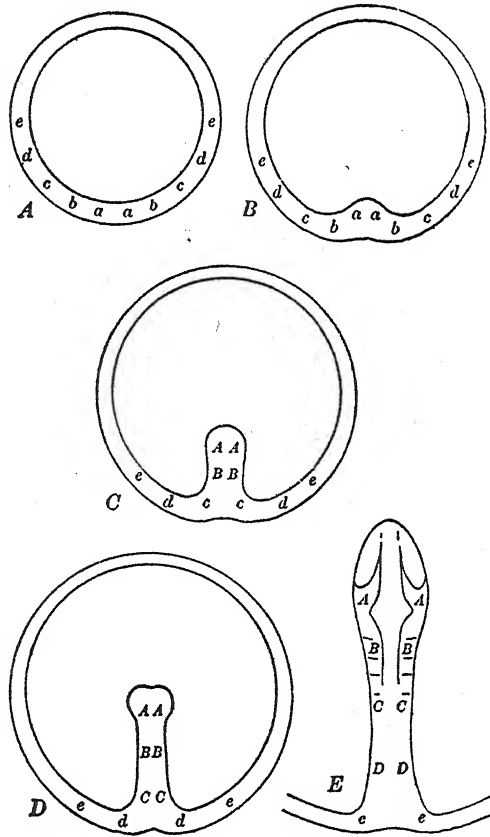


Fig. 41. — Diagram of the formation of an embryo by confluence (concrescence). From Kellicott (*General Embryology*). A. Germ ring before formation of the embryo is indicated. The letters *a-e* represent symmetrical portions of the germ ring. B. Beginning of confluence. C. Embryo forming. *AA*, *BB* represent regions of the embryo formed out of the materials of the germ ring at *aa*, *bb*. D, E. Later stages in the formation of the embryo. The germ ring regions *cc* and *dd*, have been differentiated into the embryonic regions, *CC*, *DD*.

ished. Furthermore, in the course of this procedure there is not, contrary to what might be expected, any noticeable puckering or thickening of the lips as their circumference decreases. This fact may be readily accounted for by assuming that much of the material which they contain is required to furnish the layers which they are leaving behind them. Aside from this, however, there was held to be another source for the consumption of at least part of the surplus substance of the germ

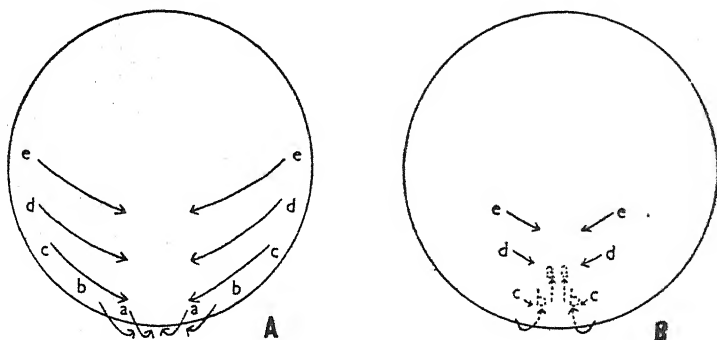


Fig. 42.—Diagrammatic representation of the process of convergence as contrasted with that of confluence or concrescence illustrated in Fig. 41. *A*. Surface view of the blastoderm at the beginning of the process. *B*. A similar view near the completion of gastrulation. Note that here most of the originally marginal material indicated by the letters, has simply moved medially and slightly posteriorly, i.e., has converged toward the median line of the future embryo and toward the dorsal blastoporal lip. Some of it represented by letters *a* and *b* has been involuted over the dorsal blastoporal lip, and hence is no longer visible. "Invisible" letters shown in dots.

ring. Thus as gastrulation proceeds it was thought that the two sides of the germ ring were flowing together at a certain point upon the margin of the blastoderm, this movement being aptly designated as *confluence* or *concrecence* (Fig. 41). In this manner, as previously noted, it was held that each side of the original ring actually came to form a lateral half of the axial structures of the embryo. Thus the halves of the ring or blastoporal lips could be thought of as the "germ" of the future embryo, and hence the name germ ring. The theory was originally applied more especially to telolecithal eggs with a very large yolk as the description and figures suggest. It was not, however, confined to these types.

The present view is that actual concrecence in the manner just described is very limited. Indeed in no case can the complete side of the axial structures of an embryo be said to arise in this manner from a half of the blastoporal rim. Actually what seems to happen in most

cases is more in the nature of a flow of material from each entire posterior half of the blastoderm toward the median line and to some extent over the dorsal blastoporal lip (involution). In this manner, much of the substance forming the axial structures of the embryo is brought into its definitive position (Fig. 42). This process of movement toward the median line may perhaps be aptly described in part as *convergence*, but hardly as concrescence in the original sense, and the latter term is now seldom used. Also in correlation with this point of view it seems scarcely appropriate any longer to speak of the blastoporal lips as the germ ring. This is because, though materials destined for certain parts do, as we have said, pass over the lips, these materials are not, to any great extent, actually furnished by them. Nevertheless the term is still employed by many embryologists especially in connection with telolecithal eggs.

FORMATION OF MESODERM AND COELOM

All animals whose tissues are formed from three fundamental cell layers are said to be *triblastic*. The Chordates belong to this group and therefore, as already indicated, possess a third embryonic layer, the *mesoderm*, which eventually lies between the other two. The source of this layer has also been mentioned, and it was stated that among Vertebrates it always arises from one or both of those previously differentiated by gastrulation. After its emergence as a separate layer the three primary layers may then, as noted, be definitely referred to as ectoderm, mesoderm and endoderm. It is now necessary to describe the ways in which mesoderm may arise. There are four chief methods, and the first is rather intimately connected with the origin of the coelom. The remaining three, as we shall see, are not quite so closely correlated.

I. The Enterocoelic Method.—This method, though common among certain Invertebrates, occurs in connection with only a few of the lowest members of the Chordate phylum. In its general aspects it may be described thus: Along each side of the archenteron in its dorsal region there arises from the hypoblast a longitudinal outpushing or fold lying between the epiblast, now ectoderm, and hypoblast, now endoderm. This is indicated diagrammatically in Figure 43, *A*. Later each fold develops a space between its two layers as shown in the diagram. Then, as a result of the downgrowth of the folds on either side, the two spaces presently meet ventrally and fuse (Fig. 43, *B*). The common cavity thus formed is the *coelom*, and its lining is mesoderm. The lining next to the ectoderm is called *somatic mesoderm*, and this somatic

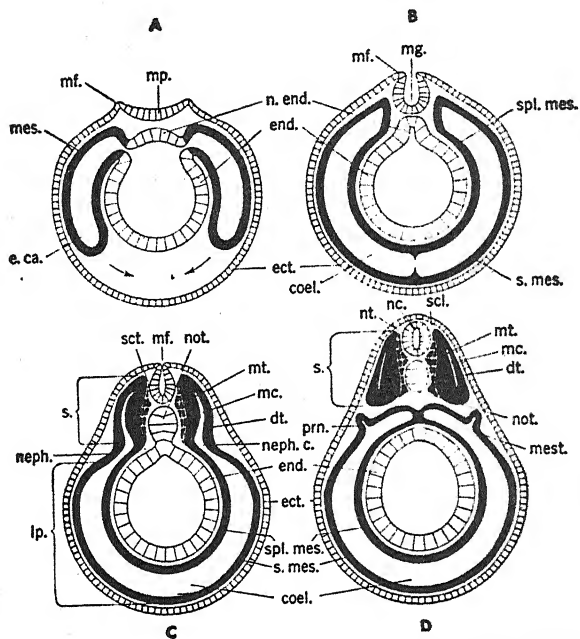


Fig. 43.—A diagram of the origin and early differentiation of the mesoderm, and of the notochord and nerve cord in a generalized Vertebrate.

A. The mesoderm is arising by means of enterocoelic pouches which are pushing out from the archenteron and are not yet separated from it. B. The enterocoelic pouches have separated from the archenteron, their walls forming the splanchnopleure and somatopleure, and their cavities the coelom. The notochord is beginning to develop and the medullary folds are approaching each other. C. The regions of the vertebral plates, which are divided transversely into somites, the nephrotomes and the lateral plates are marked out, and the various parts of the somites are distinguishable. D. The closing of the neural tube or nerve cord is completed. The somites are further developed and the myocoel is nearly obliterated. The notochord is separated from the archenteron, and the mesentery has formed. The pronephros or embryonic kidney is developing from the nephrotome.

coel. Coelom. dt. Dermatome. ect. Ectoderm. e.ca. Enterocoelic cavity. end. Endoderm. lp. Region of the lateral plate. mc. Myocoel. mf. Medullary folds. mg. Medullary groove. mp. Medullary plate. mes. Mesoderm originating in this case by the enterocoelic method. mest. Mesentery. mt. Myotome. nc. Neural canal. neph. Region of the nephrotome. neph.c. Region of the nephrocoel. not. Notochord. nt. Neural tube or nerve cord. prn. Rudiment of the pronephros or embryonic kidney. s. Region of segmental or vertebral plate (somites). scl. Sclerotome. s.mes. Somatic mesoderm. spl.mes. Splanchnic mesoderm.

mesoderm with the adjacent ectoderm are sometimes referred to together as the *somatopleure*. The lining of the coelom next to the endoderm on the other hand is called *splanchnic mesoderm*, and it together with the adjacent endoderm may be designated as *splanchnopleure*. In this case it will be noted that it was the hypoblast which gave rise to the mesoderm. Hence in this instance the hypoblast would be mesentoderm if one were using that terminology. Meanwhile dorsally the splanchnic mesoderm from either side has pressed in above the endoderm and has fused to form a double sheet of tissue called the *mesentery*. Thus the enteric canal or enteron, formally the archenteron, is, so to speak, slung from the dorsal wall of the coelomic cavity by this sheet.

It remains to be observed that, despite the rarity of this method of mesoderm formation among the Chordates, it is regarded nevertheless as of considerable zoölogical interest. The reason for this is the fact, already suggested, that it is found abundantly in some of the large Invertebrate groups (e.g., the Echinoderms and Prosopygia), and is then repeated among the lowest Chordata. This is significant because such repetition in these members of the Chordate phylum is suggestive in helping to determine from which class of Invertebrates the Vertebrate group arose.

II. The Method of Delamination.—The production of a cell layer by a method whose essential feature was a splitting off or delamination of cells has already been noted in connection with the differentiation of the first two layers. It now remains to be stated that a similar process is quite frequent among Vertebrates with respect to the generation of mesoderm. Here again the layer from which the mesoderm arises is the hypoblast, only in this case the origin is by splitting off instead of evagination (Fig. 44, *A*). Later the coelom forms by still another split within the mesoderm itself, giving rise as before to a somatic and splanchnic layer. The relations of these somatic and splanchnic layers to the body wall and to the enteron and the subsequent development of other parts are the same as in Method I.

III. The Method of Proliferation.—This method involves simply the budding off of cells from the sides of a linear thickening in the outer of the two primary layers (epiblast), along what will be the longitudinal axis of the future embryo. This thickening in these cases is termed the *primitive streak*, of which more will be said in connection with specific forms, and the cells budded from its sides soon spread out between the two primary layers, and constitute the mesoderm (Fig. 44, *B*). Presently as in the previous cases this mesoderm splits into two

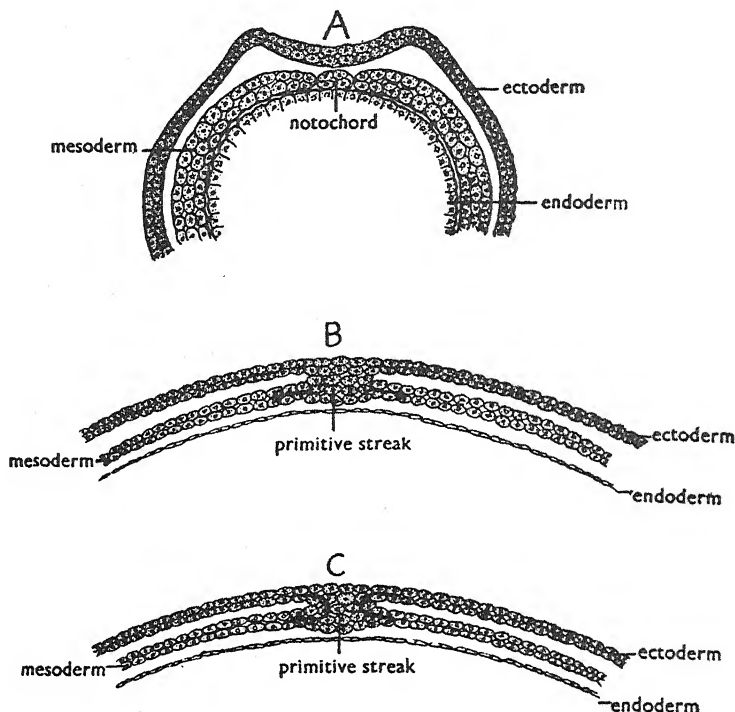


Fig. 44. — Diagrams illustrating three other methods of mesoderm origin. *A.* Method II, delamination, shows mesoderm split off from the underlying hypoblast. It is characteristic of the Frog and other Amphibians. *B.* Method III, proliferation, shows mesoderm budding off from a median longitudinal thickening of epiblast, the primitive streak. This was formerly supposed to be characteristic of the higher Vertebrates. *C.* Method IV, "invagination" or involution, shows mesoderm being involuted through the primitive streak from the overlying epiblast. This is now thought to be the method in Birds, and probably in the other higher Vertebrates. In all cases the single layer of mesoderm later splits into two with the coelom between them.

sheets. As usual the one next to the outer layer, now ectoderm, is somatic mesoderm, and that next to the inner or endodermal layer is splanchnic mesoderm with the coelom between them. It is to be noted that it is only in this last instance that the epiblast rather than the hypoblast gives rise to mesoderm. Hence on the basis of the older terminology the mesoderm in this case is mesectodermal in origin.

The method just described is one which has been supposed to prevail generally among the highest Vertebrates, i.e., the Birds and Mammals. According to the most recent evidence, however, it now seems probable that it plays little if any part in the Birds, and quite possibly this is

also true of the Mammals. Instead, considerable rather convincing evidence has been produced by Spratt, '46, in the case of one bird, the Chick, in support of a fourth process. This will be discussed in some detail in our description of mesoderm formation in that form, but to make the record complete it must be briefly indicated here.

IV. The Method of "Invagination." — This is a term which it will be recalled was used in connection with gastrulation, and indeed the process as envisaged here is essentially the same as that which is sometimes employed to describe a similar activity in endoderm production. Again as in that case, however, the writer feels that involution would be a better word to use. In fact in this case the process may be even more accurately described as a sort of combination of involution and infiltration. What is said to happen is simply this: The epiblast cells from either side of the blastoderm which aggregate along a line to form what we have designated as the primitive streak, do not remain here. Instead many of them continually move ventrad through the streak and spread out on either hand to become mesoderm (Fig. 44, C). Thus again this mesoderm might be called mesectodermal in origin.

THE SOURCES OF THE TISSUES

The three embryonic cell layers having thus been defined and their origin described, the subject may be concluded by indicating in a general way the tissues to which each cell layer eventually gives rise.

1. The ectoderm produces the epidermal part of the skin, including cutaneous glands, hair, feathers, nails, hoofs, and one type of horns and scales. It also gives rise to parts of the eye and of the internal ear, and the lining of the anus and oral cavities, including the enamel of the teeth. It is the origin of the entire nervous system and a few muscles.

2. The mesoderm gives rise to most muscles, as well as to adipose tissue and all other varieties of connective tissue including the dermis, certain types of scales and horns and the main portion (dentine) of the teeth. It also produces the skeletal system, the blood vascular system, and the greater part of the urinogenital system. It forms the coelomic epithelium, mesenteries, the outer layers of the alimentary tract, the Eustachian tube, and sometimes lines the middle ear.

3. The endoderm produces the lining of the alimentary tract and the epithelial parts of all the organs which arise as outgrowths from it; i.e., the respiratory system, the thyroid and thymus glands, the liver, and the pancreas. It also lines the middle ear in some cases, and forms a small part of the urinogenital system

THE NOTOCHORD

A characteristic feature of the embryos of all true Chordates is a rod of vacuolated tissue lying along the mid-dorsal line just above the gut. It is termed the *notochord*, and makes its appearance at about the same time at which the mesoderm starts to develop, or in some instances somewhat later. It is clearly derived in many cases from the dorsal wall of the archenteron, i.e., it is hypoblastic (Fig. 43, *B, C, D*). In some instances, however, e.g., in Birds and Mammals, the origin of the notochord is apparently partially or entirely epiblastic. The position which the structure occupies is obviously that which is taken by the vertebrae of the higher adult Chordates, i.e., the genuine Vertebrates. As will appear, the bony structures which thus replace the notochord in the latter animals arise from certain of the mesodermal tissues which surround it, while the notochord itself is gradually absorbed.

Relation of Notochord to Germ Layers.—As has been indicated, in triblastic animals all tissues and structures are supposed to be derived from one of the three primary layers. The question frequently arises therefore as to just which layer the notochord belongs. As noted it is, like the mesoderm, derived from either epiblast or hypoblast. Yet it frequently originates, in some cases partly, and in other cases entirely, separately from the mesoderm. If one is to be consistent and stick to the three-layer idea, it is probably most logical to regard the notochord as a sort of specially derived mesoderm. Otherwise it becomes a kind of embryological orphan which no layer will own. A rather common method of avoiding this dilemma of nomenclature, however, is to refer to the third layer and notochord together as *chorda-mesoderm*. Thus the intimate relation of notochord to mesoderm, as well as their semi-independent status, are both suggested in one compound term.

THE LATERAL PLATES, THE SOMITES AND THE NEPHROTOMES

Among all the Chordates, except in the case of a few of the most primitive members of the group, there accompanies or immediately follows the development of the coelom, certain other fundamental differentiations of the mesoderm. These differentiations result in the formation of three major divisions of this substance, whose origin and character may be described in a general way as follows:

I. The Lateral Plates.—It has already been suggested that the main portion of the mesoderm upon each side of the animal gives origin

LATERAL PLATES, SOMITES, NEPHROTOMES 69

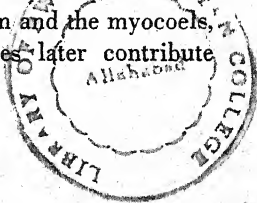
to the coelom and its lining. It remains to state that each of these portions is frequently known as a *lateral plate*.

II. The Vertebral Plates. — The mesoderm which is not involved in the production of the lateral plates, nevertheless remains connected with them for a time, lying dorsally along either side of the notochord and nerve cord in the form of a relatively narrow band, a *vertebral or segmental plate* (Fig. 43, C). The major portion of each band (i.e., all of it, save a narrow strip connecting it ventrally with the respective lateral plate) then thickens somewhat, and soon begins to be transversely divided into a series of block-like masses termed *somites*. The more anterior members of the series usually appear first, and each one as it is formed proceeds to give rise to three fundamental elements: the *dermatome*, the *myotome*, and the *sclerotome* (Fig. 43, C, D). Of these elements the relatively thin dermatomes lie next to the ectoderm, and are concerned chiefly in the production of the deeper layer of the skin, i.e., the dermis. The thicker myotomes come beneath and median to the dermatomes and give rise to the bulk of the voluntary muscles, while the sclerotomes, arising as proliferations of scattered cells, are nearest the notochord and produce the skeletogenous tissue of the axial skeleton.

It may be further remarked that in many instances at this period a small portion of the coelomic space extends up into each somite between the dermatome and myotome, and is there known as a *myocoel*. Like the connection between the somites and the lateral plates, however, it is of only temporary duration.

In *Amphioxus*, one of the very primitive Chordates referred to above, it should be noted that the term *somite* as used in the early history of this animal is somewhat more inclusive than in the foregoing description. Thus in this instance these bodies when newly formed, comprise not only the elements of the dermatomes, myotomes, and sclerotomes, but likewise those of the lateral plates. It may finally be added that since there are no bones in *Amphioxus*, the sclerotomes give rise only to connective tissue.

III. The Nephrotomes. — It will be recalled that of each band of mesoderm lying between the lateral plate and the notochord, the major dorsal portion goes to form the somites. The remaining narrow strip, which for a time connects these bodies with the corresponding lateral plate, is then designated as the *nephrotome* or *intermediate cell-mass*, while its cavity, temporarily uniting the main coelom and the myocoels, is the *nephrocoel* (Fig. 43, D). The nephrotomes later contribute chiefly to the formation of the excretory organs.



In *Amphioxus* and the other primitive Chordata no nephrotome exists, and the excretory organs are therefore of an entirely different character and origin.

THE DORSAL NERVE CORD

The final fundamental feature of Chordate anatomy which appears in connection with these very early embryonic stages is the *dorsal nerve cord* or *neural tube*. The latter term is used not only because it indicates a characteristic of this structure which is peculiar to Chordates, but also because it suggests the method of its development, which is likewise peculiar to this group. This method is as follows.

Shortly following the processes of gastrulation, and more or less concurrent with the process of mesoderm formation, a broad strip of ectoderm along the future dorsal side of the animal becomes thickened. This thickened area is termed the *medullary* or *neural plate* (Fig. 43, *A*). The median portion of this plate then becomes depressed slightly to form a groove, the *medullary* or *neural groove*, while the sides are correspondingly elevated as the *medullary* or *neural folds* (Fig. 43, *B*). These folds gradually grow toward one another until their crests meet and fuse, and there is thus developed a tube, which presently becomes entirely separated from the ectoderm above it (Fig. 43, *C, D*). This is the rudiment of the nerve cord or neural tube, while the canal which traverses its center is the *neural canal* or *neurocoel*.³ At its anterior end this canal opens to the exterior for a time through a small aperture, the *neuropore*. At the posterior end, on the contrary, the fusion of the medullary folds eliminates the external opening (except in some Sauropsids and Mammals) at an early stage, but preserves an internal passageway as follows. Instead of stopping dorsal or anterior to the nearly closed blastopore, the above folds extend slightly downward or backward upon either side of it. They then fuse above the latter orifice in such a way that through it, for a considerable time, the neurocoel communicates with the enteric cavity. The short bent portion of the neurocoel in this particular region, together with the remains of the blastopore, is then known as the *neurenteric canal* (Fig. 53).

The process thus described has already been indicated as character-

³ This method of nerve cord formation is, as noted, characteristic of most Vertebrates, but is modified somewhat in the case of the Lampreys and many of the Teleost Fishes. Thus in these animals no groove is formed in the thickening medullary plate. Instead the latter simply presses downward beneath the surface as a solid cord of tissue. The neural canal then arises later within this cord by the separation or disintegration of the cells along its axis (Fig. 144).

istic of all true Chordates, and as regards all fundamental points this is true. It should be stated, however, that once more in the case of *Amphioxus* certain minor variations occur. These will be considered in connection with the development of that animal.

REFERENCES TO LITERATURE

Abbreviations for the names of periodical publications referred to in the literature cited at the ends of chapters are as follows:

- Am. Anat. Mem. = *American Anatomical Memoirs*, Philadelphia.
 Am. Jour. Anat. = *American Journal of Anatomy*, Baltimore and Philadelphia.
 Am. Jour. Obstet. and Gynec. = *American Journal of Obstetrics and Gynecology*, St. Louis.
 Am. Jour. Physiol. = *American Journal of Physiology*, Boston.
 Anat. Anz. = *Anatomischer Anzeiger*, Jena.
 Anat. Hefte = *Anatomische Hefte*, Wiesbaden.
 Anat. Rec. = *Anatomical Record*, Philadelphia.
 Arbeit. zool. Inst. Wien. = *Arbeiten aus dem zoologischen Institute zu Wien*.
 Arch. Anat. u. Entw. = *Archiv für Anatomie und Entwicklungsgeschichte*, Leipzig. (Same as Arch. Anat. u. Physiol.)
 Arch. Anat. u. Physiol. = *Archiv. für Anatomie und Physiologie*, Leipzig.
 Arch. Biol. = *Archives de Biologie*, Leipzig and Paris.
 Arch. d'Anat. Micr. = *Archives d'Anatomie Microscopique*, Paris.
 Arch. Entw.-mech. = *Archiv für Entwicklungsmechanik der Organismen*, Leipzig.
 Arch. mikr. Anat. = *Archiv für mikroskopische Anatomie und Entwicklungsgeschichte*, Bonn.
 Arch. Zellf. = *Archiv für Zellforschung*, Leipzig.
 Arch. Zool. Exp. = *Archives de Zoologie experimentale et generale*, Paris.
 Aust. J. Exp. and Med. Sci. = *Australian Journal of Experimental Biology and Medical Science*, Sydney.
 Biol. Bull. = *Biological Bulletin*, Woods Hole, Mass.
 Biol. Centr. = *Biologisches Centralblatt*, Leipzig.
 B. M. C. Z. Harvard = *Bulletin of the Museum of Comparative Zoölogy at Harvard College*, Cambridge, Mass.
 Bull. Soc. Impér. Moscou = *Bulletins de la Societe Impériale de Natural — de Moscou*.
 Carnegie Cont. to Emb. = *Carnegie Institution. Contributions to Embryology*, Washington.
 Carnegie Inst. of Wash. = *Carnegie Institution of Washington*.
 Cold Spring Harbor Symp. on Quant. Biol. Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor.
 C. r. Soc. Biol. Paris = *Comptes rendus des séances et mémoires de la Société de Biologie*, Paris.
 Deutsche Thieraerztliche Wochenschr. = *Deutsche Thieraerztliche Wochenschrift*, Karlsruhe.
 Ergeb. Anat. u. Entw. = *Ergebnisse der Anatomie und Entwicklungsge-schichte*, Wiesbaden.
 Festsch. f. Gegenbaur = *Festschrift für Gegenbaur*, Leipzig.
 Intern. Monatsschr. = *Internationale Monatsschrift für Anatomie and Physiologie*, Leipzig.

- Jena Zeitschr. = *Jenaische Zeitschrift für Naturwissenschaft*, Jena.
 Johns Hopkins Hosp. Rep. = *Johns Hopkins Hospital Reports*, Baltimore.
 Jour. Am. Med. Assn. = *Journal of the American Medical Association*, Chicago.
 Jour. Anat. Physiol. = *Journal of Anatomy and Physiology*, London.
 Jour. Comp. Neur. = *Journal of Comparative Neurology*, Philadelphia.
 Jour. Exp. Zoöl. = *Journal of Experimental Zoölogy*, Baltimore and Philadelphia.
 Jour. Morph. = *Journal of Morphology* or *Journal of Morphology and Physiology*, Philadelphia.
 Jour. Univ. Tokyo = *Journal of the College of Science, Imperial University of Tokyo*.
 Kgl. Svensk. Vet. Handl. = *Kongliga Svenska Vetenskapsakademie, Abhandlungen aus der Naturlehre*, Leipzig.
 Mém. Acad. Impér. St. P. = *Mémoires de l'Académie Impériale de St. Petersburg*.
 Mem. Acad. roy. Belg. = *Mémoires de l'Académie royale de Belgique*.
 Mem. Boston Soc. Nat. Hist. = *Mémoires of the Boston Society of Natural History*.
 Mém. N. Y. Acad. Sci. = *Mémoires of the New York Academy of Sciences*.
 Mitt. zool. Stat. Neapel = *Mitteilungen aus der zoologischen Station zu Neapel*, Berlin.
 Morph. Arbeiten. = *Morphologische Arbeiten*, Jena.
 Morph. Jahrb. = *Morphologisches Jahrbuch*, Leipzig.
 Nat.-wiss. = *Die Naturwissenschaften*, Berlin.
 Phil. Trans. Roy. Soc. = *Philosophical Transactions of the Royal Society of London*.
 Physiol. Rev. = *Physiological Reviews*, Baltimore.
 Physiol. Zoöl. = *Physiological Zoölogy*, Philadelphia.
 Proc. Am. Acad. Arts and Sci. = *Proceedings of the American Academy of Arts and Sciences*, Boston.
 Proc. Am. Phil. Soc. = *Proceedings of the American Philosophical Society*, Philadelphia.
 Proc. Internat. Cong. Zoöl. Cambridge = *Proceedings of the International Congress of Zoölogists*, Cambridge.
 Proc. Soc. Exp. Biol. and Med. = *Proceedings of the Society for Experimental Biology and Medicine*, New York.
 Proc. Zoöl. Soc. = *Proceedings of the Zoölogical Society of London*.
 Q. J. M. S. = *Quarterly Journal of Microscopical Science*, London.
 Quart. Rev. Biol. = *Quarterly Review of Biology*, Baltimore.
 S. B. G. M. P. = *Sitzungs-Berichte der Gesellschaft für Morphologie und Physiologie*, München.
 Sitzber. Ber. Akad. = *Sitzungsberichte der Koeniglich Preussischen Akademie der Wissenschaft*, Berlin.
 Tijd. Nederl. dierk. Ver. = *Nederlandsche dierkundige Vereeniging*, Tijdschrift, Leyden.
 Trans. Am. Phil. Soc. = *Transactions of the American Philosophical Society*, Philadelphia.
 Univ. Cal. Press. = *University of California Press*, Berkeley.
 Verh. d. Anat. Gesell. = *Verhandlungen der Anatomischen Gesellschaft*, Jena.
 Ver. kon. Akad. Wetensch. = *Verhandelingen koninklijke Akademie van Wetenschappen*, Amsterdam.
 Verh. Phys.-Med. Ges. = *Verhandlungen Physikalische-Medizinische Gesellschaft*, Wurzburg.

- Zeit. Anat. Entw. = *Zeitschrift für Anatomie und Entwicklungsgeschichte*, Leipzig.
 Zeit. ind. Abs. u. Vererb. = *Zeitschrift für induktive Abstammungs- und Vererbungslehre*, Berlin.
 Zeit. Mikr.-Anat. Forsch. = *Zeitschrift für Mikroskopisch-Anatomische Forschung*, Leipzig.
 Zool. Jahrb. = *Zoologische Jahrbücher*, Jena.

CHAPTERS I AND II

- Allen, B. M., "The Origin of the Sex-Cells of Chrymys," *Anat. Anz.*, XXIX, 1906.
 Allen, Edgar, "Ovogenesis during Sexual Maturity," *Am. Jour. Anat.*, XXXI, 1923.
 Allen, E., Kountz, W. B. and Francis, B. F., "Selective Elimination of the Ova in the Adult Ovary," *Am. Jour. Anat.*, XXXIV, 1925.
 Babcock, E. B. and Clausen, R. E., *Genetics in Relation to Agriculture*, New York and London, 1918.
 Benda, C., "Die Mitochondria," *Ergeb. Anat. u. Entw.*, XII, 1903 (1902).
 Bookhout, C. G., "The Development of the Guinea Pig Ovary from Sexual Differentiation to Maturity," *Jour. Morph.*, LXXVII, 1945.
 Boveri, Th., "Die Entstehung des Gegansatzes zwischen den Geschlechtszellen und den somatischen Zellen bei Ascaris," *S.B.G.M.P.*, München, VIII, 1895.
 Bowen, R. H., "Studies on Insect Spermatogenesis," VI, "Notes on the Formation of the Sperm in Coleoptera and Aptera, with a General Discussion of Flagellate Sperms," *Jour. Morph. and Physiol.*, XXXIX, 1924.
 Bütschli, O., *Untersuchungen über mikroskopische Schäume und das Protoplasma*, Leipzig, 1892.
 Castle, W. E., *Genetics and Eugenics*, 2nd Ed., Harvard Univ. Press. 1920.
 Everett, N. B., "The Origin of Ova in the Adult Opossum," *Anat. Rec.*, LXXXII, 1942. — "Observational and Experimental Evidences Relating to the Origin and Differentiation of the Definitive Germ Cells in Mice," *Jour. Exp. Zool.*, LIXII, 1943.
 Flemming, W., *Zellsubstanz, Kern und Zellteilung*, Leipzig, 1882.
 Geerts, J. M., "Cytologische Untersuchungen einiger Bastarde von *Oenothera gigas*," *Berichte Deutsche Botanische Gesellschaft*, XXIX, 1911.
 Goldsmith, J. B., "The History of the Germ Cells in the Domestic Fowl," *Jour. Morph. and Physiol.*, XLVI, 1928.
 Goodrich, H. B., "The Germ Cells in Ascaris," *Jour. Exp. Zool.*, XXI, 1, 1916.
 Hargitt, G. T., "The Formation of the Sex Glands and Germ Cells of Mammals." I. "The Origin of the Germ Cells in the Albino Rat," *Jour. Morph. and Physiol.*, XL, 1925. — II. "The History of the Male Germ Cells in the Albino Rat," *Jour. Morph. and Physiol.*, XLII, 1926. — III. "The History of the Female Germ Cells in the Albino Rat to the Time of Sexual Maturity." — IV. "Continuous Origin and Degeneration of Germ Cells in the Female Albino Rat," *Jour. Morph. and Physiol.*, XLIX, 1930.
 Hertwig, A., *Die Zelle und die Gewebe*, Jena, I, 1893; II, 1898.
 Holtfreter, J., "A Study of the Mechanics of Gastrulation," Part I, *Jour. Exp. Zool.*, VIC, 1943. — Part II, *Jour. Exp. Zool.*, VC, 1944.
 Humphrey, R. R., "The Primordial Germ Cells of Hemidactylum and other Amphibia," *Jour. Morph. and Physiol.*, XLI, 1925. — "Extirpation of the Primordial Germ Cells of Amblystoma: Its Effect Upon the Development of the Gonad," *Jour. Exp. Zool.*, XLIX, 1927. — "The Early Position of the Primordial Germ Cells in Urodeles: Evidence from Experimental Studies," *Anat. Rec.*, XLII, 1929.

- Jenkinson, J. W., "Observations on the Maturation and Fertilization of the Egg of the Axolotl," *Q.J.M.S.*, XI, viii, 1904. — *Vertebrate Embryology*, Oxford and London, 1913.
- Jennings, H. B., "Paramecium bursaria. Life History. V. Some Relations of External Conditions, Past or Present, to Aging and to Mortality of Ex-conjugants, with Summary of Conclusions on Age and Death," *Jour. Exp. Zool.*, IC, 1945.
- Kingsbury, B. F., "The Postpartum Formation of Egg Cells in the Cat," *Jour. Morph.*, LXIII, 1938.
- Lewis, W. H., "Mechanics of Invagination," *Anat. Rec.*, IIIC, 1947.
- Lillie, F. R., *Problems of Fertilization*, Chicago, 1919.
- McClung, C. E., "The Accessory Chromosome — Sex Determinant?" *Biol. Bull.*, III, 1902.
- Meves, F., "Ueber Struktur und Histogenese der Samenfäden von Salamandra," *Arch. mikr. Anat.*, I, 1897.
- Moenkhaus, W. J., "The Development of the Hybrids between *Fundulus heteroclitus* and *Mendidia notata*, with Special Reference to the Behavior of the Maternal and the Paternal Chromatin," *Am. Jour. Anat.*, III, 1904.
- Montgomery, T. H., Jr., "A Study of the Chromosomes of the Germ Cells of the Metazoa," *Trans. Am. Phil. Soc.*, XX, 1901. — "On the Dimegalous Sperm and the Chromosomal Variation of *Euschistus* with Reference to Chromosomal Continuity," *Arch. Zellf.*, V, 1910.
- Morgan, T. H., *Heredity and Sex*, New York, 1913. *The Physical Basis of Heredity*, Philadelphia, 1919. *The Physical Basis of Heredity*, Philadelphia and London, 1919. *The Theory of the Gene*, Yale Univ. Press, 1926.
- Morgan, Sturtevant, Muller, and Bridges, *The Mechanism of Mendelian Heredity*, New York, 1915.
- Oliver, J. R., "The Spermiogenesis of the Pribilof Fur Seal (*Callorhinus alascanus* J. and C.)," *Am. Jour. Anat.*, XIV, 1913.
- Painter, T. S., "Studies in Mammalian Spermatogenesis. II, The Spermatogenesis of Man," *Jour. Exp. Zool.*, XXXVII, 1923.
- Riddle, O., "The Theory of Sex as Stated in Terms of Results of Studies on Pigeons," *Science*, XLVI, 1917.
- Rosenberg, O., "Cytologische und morphologische Studien an *Drosera longifolia* × *rotundifolia*," *Kgl. Svensk. Vet. Handl.*, 43, 1909.
- Sharp, L. W., *An Introduction to Cytology*, New York, 1921.
- Sinnott and Dunn, *Principles of Genetics*, New York, 1925.
- Sneider, M. E., "Rhythms of Ovogenesis before Sexual Maturity in the Rat," *Am. Jour. Anat.*, LXVII, 1940.
- Strassburger, E., *Zellbildung und Zellteilung* (3rd ed.), Jena, 1880.
- Sutton, W. S., "On the Morphology of the Chromosome Group in *Brachystola magna*," *Biol. Bull.*, IV, 1902.
- Van Beneden, E., "Recherches sur la Composition et la Signification de l'Œuf etc.," *Mem. Acad. roy. Belg.*, XXXIV, 1870. — "Recherches sur la Maturation de l'Œuf et la Fécondation," *Arch. Biol.*, IV, 1883.
- Weismann, A., "Entstehung der Sexualzellen bei den Hydromedusen," Fischer, Jena, 1885.
- Wilson, E. B., *Atlas of Fertilization and Karyokinesis*, New York, 1895. — *The Cell in Development and Heredity* (Columbia University Biological Series, IV, 3rd ed.), New York, 1925. — "Studies on Chromosomes," *Jour. Exp. Zool.*, XIII, 3, 1912.



THE EARLY DEVELOPMENT OF AMPHIOXUS

THE early stages in the development of *Amphioxus* (*Branchiostoma lanceolatum*) are taken up because in this form these stages are thought to be as nearly primitive as those occurring in any other Chordate. This applies particularly to the method of segmentation, gastrulation, and formation of the mesoderm and coelom. Indeed the general resemblance of these processes to what occurs among Invertebrates, such as the Echinoderms, is so marked that their primitive character in *Amphioxus* can hardly be doubted. Also according to the most recent studies there is a marked and significant resemblance between the early stages of this animal and those forms sometimes designated as Protochordates, i.e., the Ascidians.

There are numerous accounts of the development of this classic form, some of the best known being those of Hatschek (1882, '88), Wilson (1893), Cerfontaine ('06) and the most recent that of Conklin ('32). The studies of the last named investigator, though agreeing in many respects with those of his predecessors, differ rather fundamentally in some of the earlier details. Since the work of Conklin is not only the most recent, but is supported both by elaborate observations of normal development, and by experimental procedures, it is believed to be the most accurate. It is therefore the one followed in this text except where otherwise indicated. It is assumed that the student has in mind a fair knowledge of the adult anatomy of the animal under discussion.

THE REPRODUCTIVE ORGANS

THE OVARY

Since the work of Conklin does not cover very completely the character of the ovary and the process of oögenesis the following brief statements on these subjects are based on the account of Cerfontaine.

The ovaries are developed in each myocoel (Fig. 45) on both sides of the body from the tenth to the thirty-fifth or thirty-sixth somite inclusive. Each originates as a proliferation of cells on the antero-ventral

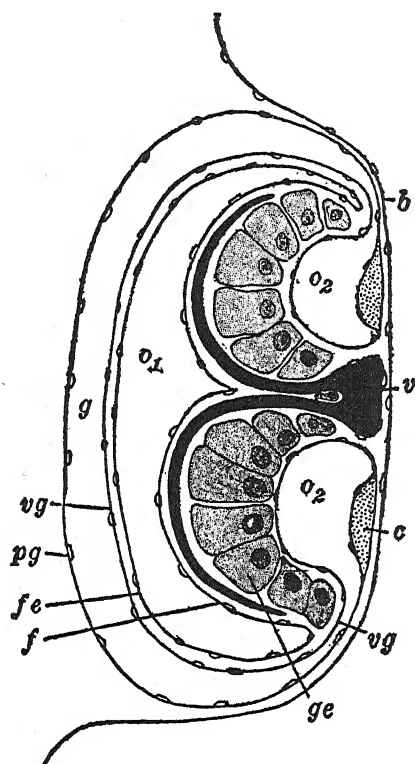


Fig. 45.—Diagram of a section through the gonad of *Amphioxus*. From Kellicott (*Chordate Development*). After Cerfontaine. Right side adjacent to atrium.

b. Peribranchial (atrial) epithelium. *c.* Cicatrix. *f.* True follicular epithelium. *fe.* External layer of follicular epithelium. *g.* Gonocoel. *ge.* Germinal epithelium. *o₁.* Primary ovarian cavity. *o₂.* Secondary ovarian cavity. *pg.* Parietal layer of gonocoel. *v.* Cardinal vein. *vg.* Visceral layer of gonocoel.

while the ovary during the process almost disappears. It then develops anew in preparation for the next breeding season.

THE TESTIS

The development of the testes in *Amphioxus* is not so well known, but it appears to be similar in a general way to that of the ovary. The products are discharged to the outside as are the eggs.

wall of the myocoel. This proliferation then pushes forward as a small bud, covered by the portion of the myocoelic wall from which it arose. The bud of germ cells with its covering thus comes to project sac-like into the myocoel anterior to the one in which the proliferation started. The neck of the sac then forms a short stalk connecting it with the posterior myocoelic wall of the cavity into which the evagination has occurred. Thus in these animals each egg is not surrounded by its individual follicle, but is attached to the wall of the above sac, which acts as a general follicle for all the ova within it. As development proceeds, the most ventral part of each myocoel which contains the gonad is cut off from the part above as the *gonocoel*. By the time a batch of ova is ripe, however, which occurs for the first time in animals about two centimeters in length, each ovary has grown so that it virtually obliterates all coelomic spaces surrounding it (Fig. 45). These eggs are then extruded (see below),

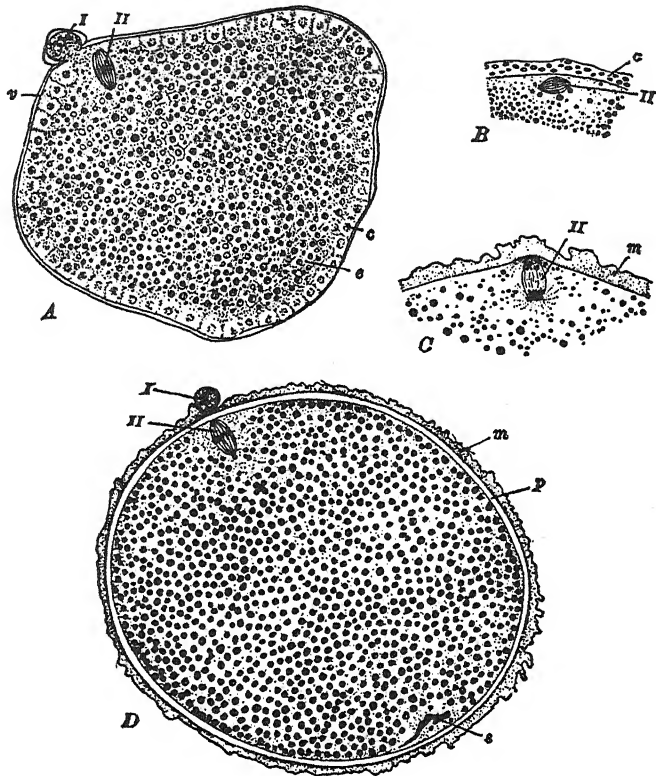


Fig. 46.—The egg of *Amphioxus*. From Kellicott (*Chordate Development*). C. After Cerfontaine, others after Sobotta. A. The ovarian egg showing cortical plasm. The first polar body is being pinched off, and the spindle for the second meiotic division is formed. B. The cortical layer forming the perivitelline membrane on the surface of the egg within the vitelline membrane. C. The fusion of the vitelline membrane and perivitelline membrane to form the fertilization membrane is complete, but the latter has not yet left the surface of the egg. D. The extruded and fertilized egg. The fertilization membrane is beginning to leave the surface of the egg. c. Cortical layer. e. Endoplasm. m. Fused vitelline and perivitelline membranes, i.e., the fertilization membrane. p. Perivitelline space. s. Spermatozoön. v. Vitelline membrane. I. First polar body. II. Second polar spindle.

THE HISTORY OF THE OVUM TO GASTRULATION
OÖGENESIS

Multiplication and Growth.— After passing through a typical oögonial or multiplication stage the cells cease dividing and enter upon a period of growth. During this period the nucleus passes through the last processes prior to meiosis, while deutoplasm appears throughout the greater part of the cytoplasm. Inasmuch as this is a comparatively yolk-free egg the latter substance does not become very dense. It does become just abundant enough, however, so that the yolkless portion is clearly distinguishable. At the conclusion of growth and previous to the maturation divisions this portion apparently consists mainly of a thin vacuolated layer lying everywhere just beneath the surface (Fig. 46, *A*). The germinal vesicle is in contact with this layer on one side, the animal pole, while the remainder of the egg cytoplasm is relatively full of yolk granules. Near the close of the growth period a thin vitelline membrane is formed.

MATURATION AND FERTILIZATION

The First Meiotic Division.— When the egg has reached full size the first meiotic division takes place at the animal pole. It is preceded in this instance by the formation of tetrads (see page 20), and the spindle of this and the ensuing division are without centrosomes or asters. Immediately following this division, preparations for the second one begin, and proceed as far as the metaphase (Fig. 46, *A*). The process pauses in this stage until after fertilization. Meanwhile as the first polar body separates from the egg it pushes through the vitelline membrane, carrying a small portion of the latter with it. Hence it is entirely free and is often lost (Fig. 46, *D*). At the same time the egg bursts out into a portion of the gonocoel next to the atrium.

Spawning and Fertilization.— Spawning occurs throughout the spring and summer, and always toward evening, while the animals are swimming. At this time muscular contractions occur in the walls of the above gonocoel cavities and thus cause the eggs to burst through these walls, at certain points termed the *cicatrices*. The cutis wall of the atrium is also ruptured in these regions and the eggs thus reach the atrial cavity and from thence the exterior. As soon as the egg comes in contact with the sea water a second membrane is formed inside the first. It is called the *perivitelline membrane*, and is separated from the origi-

nal covering by a slight space.¹ The new membrane seems to be formed from the outer part of the vacuolated cytoplasm (cortical plasm) at the surface of the ovum, with which for a short time it remains in close contact. It is at first of a fluid consistency, but after a brief exposure to the action of the water it begins to toughen. This process starts in the

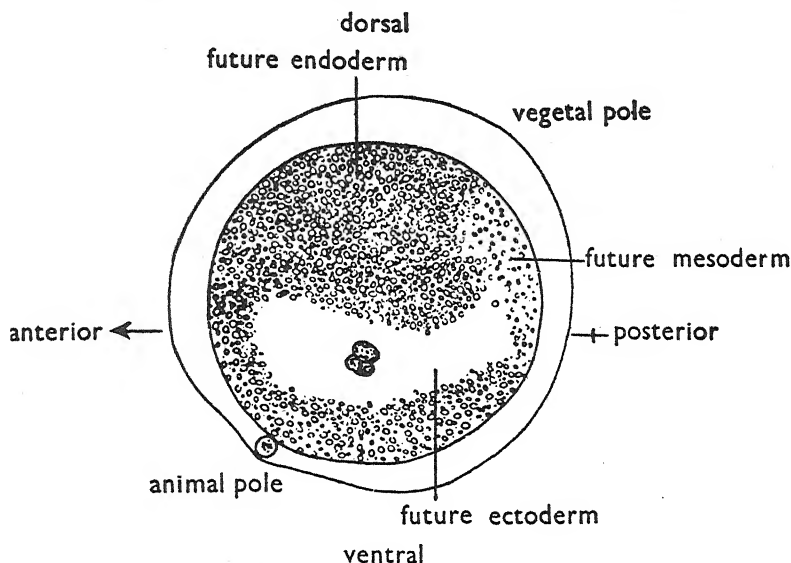


Fig. 47.—A median sagittal section through the fertilized egg of *Amphioxus*, viewed from the left side. After Conklin. The egg is oriented in terms of the position of its parts relative to the future embryo. Actually, according to Conklin, it floats with the animal pole up at this time. The fertilization membrane is shown at some distance from the egg, and beneath it at the animal pole is the second polar body. The pronuclei are shown fusing in the midst of the clear hyaloplasm.

region of the animal pole, from where it soon spreads rapidly around the egg.

Meanwhile the latter has become surrounded by sperm which have been shed into the water near the female. One or more of these sperm now penetrate the outer or vitelline membrane, cross the intervening space, pierce the inner membrane, and enter the egg. Such entrance is generally effected near the vegetal pole where the perivitelline covering remains longest in a fluid condition. As soon as the sperm have reached the egg itself, however, the toughening of this membrane is rapidly com-

¹ This space is literally perivitelline, and is often referred to as such. It differs from the space more usually so named, however, in that it exists previous to fertilization, and also in that it is, at this time, separated from the egg by a separate covering, the perivitelline membrane.

pleted. Also it seems to fill the space between the egg and the original vitelline membrane with which it apparently becomes fused (Fig. 46, B, C). The fused membranes thus form together what may be termed a *fertilization membrane*, and this presently becomes separated from the surface of the egg by the usual ("true") perivitelline space (Fig. 46, D).

The Second Meiotic Division: Fusion of the Egg and Sperm Nuclei.—The entrance of the sperm is a stimulus which causes the second meiotic division to become completed, and the second polar body is cut off. In this case, however, the body is retained beneath the fertilization membrane, thus helping to mark the animal pole, and so to orient the egg.

Meanwhile the sperm head (i.e., the sperm nucleus) enlarges so that it is equal in size to the egg nucleus. The two nuclei then meet and fuse in the usual manner. The point of this fusion is generally a little above the equator of the egg, and slightly toward the side which will eventually be the posterior of the embryo, as shown in Figure 47. As is indicated in this figure, the fused nuclei now lie within an area of clear cytoplasm (hyaloplasm) which, though it is mainly toward the animal pole, extends somewhat posteriorly. Cerfontaine represents it as a cone as outlined in Figure 48, A, though Conklin (Fig. 47) shows this shape less clearly. The source of this hyaloplasm is not quite clear, though Conklin seems to suggest that it arises from the breakdown of the germinal vesicle, at the maturation divisions. Whatever its source this clear material should be noted as the third differentiated substance in the unsegmented egg, the other two being the yolk filled cytoplasm, and the peripheral vacuolated layer. The further fate of these substances will be indicated presently. Any other sperm which may have gained entrance degenerate without further activity and the process of fertilization may be said to be complete.

EGG SYMMETRY AND SEGMENTATION

Symmetry and Orientation.—The polarity of the egg, i.e., the establishment of the animal and vegetal poles, is traceable to its point of attachment in the ovary; i.e., the vegetal pole is on the unattached side. This is a matter of considerable interest because, as Conklin has pointed out, in many Invertebrates it is the vegetal pole which is attached in the ovary. This writer then very pertinently suggests that this reversal may well mark the initiation of the later reversal in dorso-ventral symmetry which places the nerve cord in Chordates on the dor-

sal instead of the ventral side. This seems reasonable, since such a profound and early appearing difference as this must certainly have its origin very far back in the ontological process.

Whatever may be the conclusion with respect to this question, it is evident that the entrance of the sperm slightly to one side of the vegetal

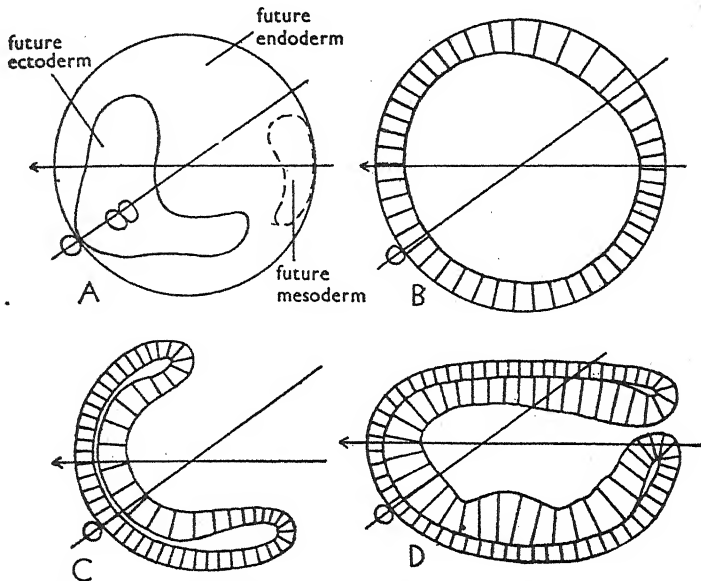


Fig. 48. — Diagrams illustrating the relations between the adult axes and the axes of the egg and early stages based on the accounts by Cerfontaine and Conklin. *A*. Fertilized egg. *B*. Fully formed blastula. *C*. Gastrulation begun. *D*. Fully formed gastrula. The arrow in each case indicates the future anterior-posterior axis, while the polar body marks the animal pole. In *A* the pronuclei are represented as fusing in the midst of the cone of yolk-free cytoplasm. (See Fig. 47.) According to Conklin, the egg or embryo does not actually assume the position indicated until shortly after the closure of the blastopore (see text).

pole establishes a third point on the egg with reference to the two poles already present, and so determines a median plane. Not only is this true but, as later events prove, this median plane of the egg becomes the median plane of the future embryo, and the side toward which the sperm enters becomes the posterior side of the embryo. This is well to bear in mind since in the study of the Frog we shall find another case in which the sperm entrance point is significant in determining embryonic symmetry.

With respect to this matter of embryonic symmetry, a further word

must now be said. Though the bilateral and antero-posterior symmetries of the future embryo have now been determined in the egg as indicated, the question arises as to how soon the floating egg or developing embryo actually becomes oriented with the antero-posterior and dorso-ventral parts in their definitive positions. It has been said that this occurs at the time of, or immediately following, fertilization so that the undivided egg actually assumes an orientation in the water such as indicated in Figures 47 and 48, *A*. As a matter of fact, however, this appears probably not true. Conklin does not refer to the point in his paper, but has been kind enough to inform the writer that in his opinion this definitive orientation probably does not occur until "shortly after the closure of the blastopore." In the meantime this investigator believes that the dividing egg probably floats like most other floating eggs, with the animal pole up. The lack of certainty in this connection is due, Conklin says, to the fact that "the polar bodies are minute and difficult to recognize," while other means of orientation are also hard to discern in the living egg. His opinion under these circumstances is based on such observations as are possible, and on the fact that on the centrifuge the yolk pole always goes to the centrifugal position. However, in spite of this probable actual orientation of the animal and vegetal poles of the egg, it is convenient in describing development to assume a constant orientation from the very beginning. Hence in the ensuing description the terms dorsal, ventral, anterior, and posterior are used throughout with reference to the definitive position of these parts subsequent to gastrulation. This relation of the animal and vegetal poles of the egg to the orientation of the future embryo is indicated in Figure 48. On this basis it is evident that the anterior end of the future animal will lie about 30 degrees above the animal pole of the egg as here shown and the posterior of the animal about 30 degrees below the vegetal pole. It is to be borne in mind, however, that according to Conklin the developing ovum probably does not really assume this position until about the stage represented by Figure 51, *F*, or shortly thereafter.

In addition to the plane of symmetry established by the mere entrance of the sperm and the position of the fusion nucleus, other significant reinforcements of the symmetry so initiated quickly ensue. As the sperm passes into the egg there is, according to Conklin, a flow of the superficial vacuolated layer of cytoplasm from the animal pole to the region where the spermatozoon entered. Here it forms a crescent of material across the future posterior surface of the egg, as above de-

fined, with the horns of the crescent extending somewhat anteriorly. This, Conklin emphasizes, is exactly comparable to the mesodermal crescent similarly formed in the Ascidians, and it has exactly the same fate, i.e., it gives rise to all the future mesoderm. This conclusion is based on a study of sections of successive stages, the flow not being actually observed in the case of *Amphioxus*. Aside from the potential

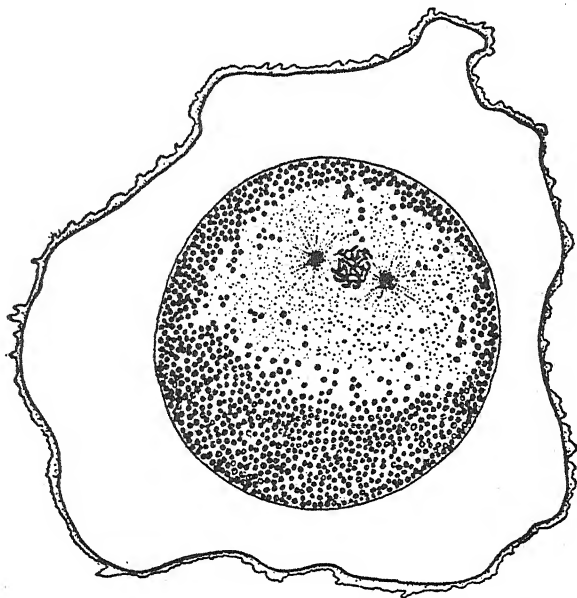


Fig. 49. — Prophase of first cleavage figure in *Amphioxus*. From Kellicott (*Chordate Development*). After Sobotta. Inner and outer membranes fused and separated from the egg by a wide space called the perivitelline space.

mesodermal material which Conklin thus finds preformed in the egg, this investigator also noted that the future endodermal substance consists of the yolk-filled cytoplasm now located dorso-anteriorly to that destined to be mesoderm. The remaining yolk-free cytoplasm or hyaloplasm, containing the cleavage nucleus then lies, as noted, largely toward the antero-ventral side, and is destined to become ectoderm and notochord (Figs. 47, 48). We are now prepared to describe the process of segmentation, keeping always in mind the sense in which dorsal, ventral, anterior, and posterior are being used.

Segmentation.—Segmentation in *Amphioxus* is of the total or holoblastic type, but is not quite equal. The first division occurs about

84 THE EARLY DEVELOPMENT OF AMPHIOXUS

an hour after fertilization, and the second about an hour after the first. Subsequent divisions follow each other at intervals of fifteen or twenty minutes.

First Cleavage. — The first cleavage spindle becomes situated within the cone of clear protoplasm, where its position is such that its center is cut at right angles by the median plane of the egg. The line of cleavage, therefore, coincides with that plane, and divides the ovum, including its three preformed substances, into equal right and left halves (Fig. 49).

Second Cleavage. — The second cleavage is at right angles to the first, and is also approximately meridional. It is not exactly so, however, since its plane lies a little postero-ventral to the animal and vegetal poles, thus causing the antero-dorsal pair of blastomeres to be slightly larger than the postero-ventral pair (Fig. 50, *A*). This is the interpretation of Conklin, and is exactly the opposite of that of Cerfontaine and others. It is significant because it carries through the entire early development, and is necessary in order to locate the potential mesoderm in the ventro-lateral lips of the early gastrula where Conklin insists it is. We shall follow Conklin's interpretation. This writer also calls attention to a slight spiral tendency in this cleavage comparable to what regularly occurs in Annelids and Gastropods. He maintains that usually two of the four blastomeres are sufficiently in apposition so that when viewed from the animal pole the line of contact at that pole appears as a short furrow turning to the left. From the same viewpoint the furrow at the vegetal pole turns to the right. This feature, however, does not have the constancy which is characteristic of the Invertebrate forms referred to.

Third Cleavage. — The third cleavage plane is at right angles to the first two; i.e., it is latitudinal with respect to the animal and vegetal poles of the egg. It is not quite equatorial, however, since it is situated slightly nearer the animal pole. The result is the production of four pairs of cells, the two at the animal pole being termed *micromeres*, and the two at the vegetal pole *macromeres* (Fig. 50, *B, C*). As regards the orientation of these cells relative to the future embryo, the upper pair of micromeres are anterior, and the lower pair ventral, while the upper pair of macromeres are dorsal and the lower pair posterior. From the account of the preceding cleavage also it is evident that the anterior pair of micromeres and the dorsal pair of macromeres are respectively slightly larger than the other pair of the same type. Likewise it is to be noted that the potential mesodermal material is largely located in

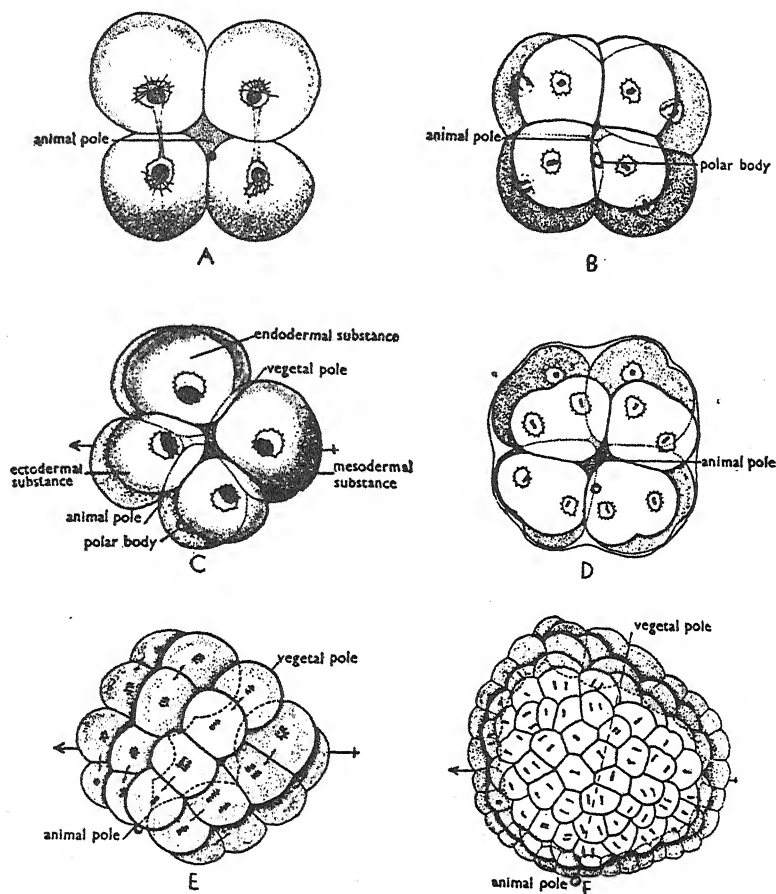


Fig. 50. — Cleavage in *Amphioxus*. After Conklin. *A*. Four-cell stage viewed from the animal pole. *B*. Eight-cell stage viewed from the animal pole, showing the four sizes of the cells. *C*. Eight-cell stage viewed from the left side. The arrow indicates the anterior-posterior axis. Again note the relative sizes of the cells, the anterior micromeres being slightly larger than the ventral ones, and the dorsal macromeres slightly larger than the posterior ones which contain the mesodermal substance. *D*. Eight-cell stage going into sixteen viewed from the animal pole. *E*. Thirty-two cell stage viewed from the left side with many of the cells about to divide again. The arrow indicates the anterior-posterior axis. *F*. About the 128-cell stage, four hours after fertilization, viewed from the left side. The arrow indicates the anterior-posterior axis. Note that at this time the largest of all the cells are at the future dorsal blastoporal lip, and represent the endoderm. (See Fig. 51, *A*.)

86 THE EARLY DEVELOPMENT OF AMPHIOXUS

the two posterior (smaller) macromeres, the potential endodermal material in the dorsal macromeres and the dorsal parts of the posterior macromeres, and the potential ectodermal substance chiefly in the micromeres (Fig. 50, C).

Fourth Cleavage.—The planes of this cleavage are again approximately longitudinal or meridional with respect to the poles of the egg. The cleavage is not precisely meridional, however, in all the blastomeres but very slightly bilateral. Thus in the four micromeres each of the new planes runs not exactly toward the center of the egg, but a little toward the plane of the first cleavage, while in the macromeres the inclination of these fourth cleavage planes is a little toward the plane of the second cleavage. This may be noted to some extent in Figure 50, D, although the incipient planes of the macromeres in this case appear to be essentially meridional.

Fifth Cleavage.—This division is typically again latitudinal, so that there result two layers of micromeres and two of macromeres. Thus, in all, there are thirty-two cells arranged in eight meridional rows with respect to the original animal and vegetal poles, the micromeres toward the former and the macromeres toward the latter. It should be added, however, that the arrangement of the cells following this cleavage is seldom entirely regular so that a strictly meridional appearance such as shown in Figure 50, E is not often seen.

The Blastula.—The sixth cleavage is more or less meridional, giving rise to sixty-four cells. The arrangement is even more irregular than in the last case, however, and it is impossible to identify exactly the various cells in terms of their origins. Although the seventh cleavage is more irregular than the sixth, about one hundred twenty-eight cells are produced, and by the eighth cleavage the synchronous character of the divisions is also lost. This dividing mass of cells may now be termed a *blastula* (Fig. 50, F). From the figure just referred to it will be evident that this blastula is not round. Instead it is somewhat pear-shaped with the small end of the pear posterior. Also, as might be assumed, it is not a solid mass of cells, but as usual contains a cavity or *blastocoel*. This indeed has existed from the four cell stage since the cells are rounded and hence not in complete contact. The space in question is filled with a gelatinous material which Conklin calls *blastocoel jelly*, and at first communicates with the outside through spaces between the rounded cells. As cleavage continues, however, the cells establish contacts except at their inner ends, and thus close the openings into the blastocoel, the ones at the poles persisting longest. Meanwhile the jelly

in the blastocoel is absorbing water, so that it greatly increases in volume, and becomes quite fluid. As a result of this increase in volume the size of the completed blastula is about one third greater than that of the unsegmented egg.

The fact that the cells of the blastula are somewhat irregularly arranged makes it, as noted, almost impossible to identify each one precisely in terms of its source. Nevertheless this relationship can be approximately determined by the positions of the cells with respect to the polar body, and by their relative sizes. Thus it appears that the smallest and most rapidly dividing cells of the blastula are located posteriorly. Hence they are derived from the two posterior macromeres of the eight cell stage, and represent potential mesoderm. The somewhat larger and slightly more slowly dividing cells located in the antero-ventral region are derived from the four micromeres of the eight cell stage, and are potential ectoderm. Finally the largest and most slowly dividing cells in the postero-dorsal region are derived mostly from the dorsal pair of macromeres of the eight cell stage and are potential endoderm (Fig. 50, *F*).

GASTRULATION, FORMATION OF CENTRAL NERVOUS SYSTEM, MESODERM, NOTOCHORD, AND COELOM

GASTRULATION

The exact nature of the process of gastrulation in *Amphioxus* has been the subject of much dispute. This is owing partly at least to the minute size of the larva at this time, and the consequent difficulty of determining just what occurs. As before, the account which will be followed here is that of Conklin, according to whom the main processes are invagination, involution and a kind of epiboly. It should be stated, however, that Conklin does not himself employ the last named term. Concrescence, which is said to occur by Cerfontaine and other writers, is, according to this investigator entirely lacking. Conklin indeed does not even refer to convergence.

Invagination and Involution. — As noted the completed blastula consists of a hollow pear-shaped mass of cells the wall of which is everywhere a single cell layer in thickness. Antero-dorsally from the smaller posterior end of this pear shaped structure, the hypoblastic wall, consisting of potential endodermal cells, is already somewhat flattened (Fig. 51, *A*), and this process soon involves the whole postero-dorsal

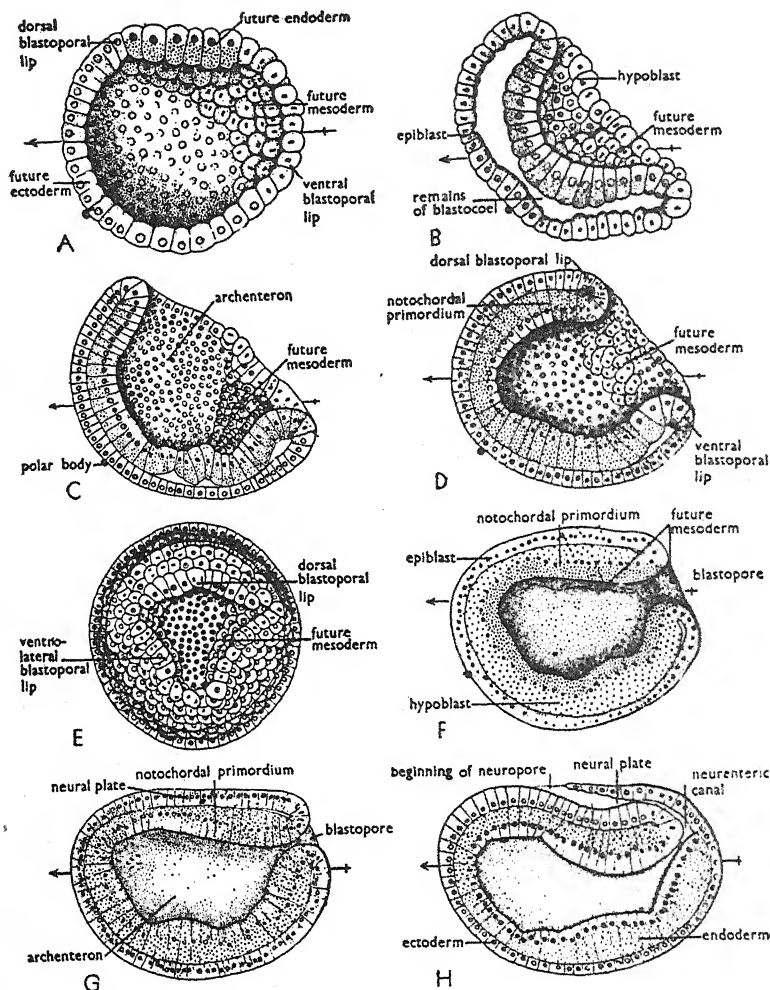
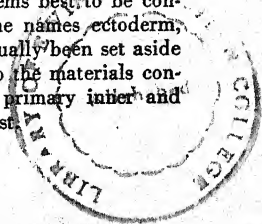


Fig. 51.—Gastrulation in *Amphioxus*. After Conklin. Arrows indicate anterior-posterior axis. *A*. Hemisected blastula from left (cut) side. Note flattened vegetal pole preliminary to gastrulation, also position of future endoderm and mesoderm. *B*. Moderately early hemisected gastrula from left side with epiblast of right side removed, permitting view through remains of blastocoel. *C*. Slightly later gastrula. Same view and treatment as in *B*. Note position of future mesoderm. *D*. Still later gastrula. Same view and treatment as in *B* and *C*. Note posterior movement of dorsal lip and dorsal movement of ventral lip, thus bringing mesoderm nearer to dorsal lip. *E*. Posterior view of total gastrula slightly later than *D*. Future mesoderm apparent in both lateral lips, but not in ventral lip, though it is there. (See text.) *F*. Much later hemisected gastrula, again viewed from left (cut) side. *G*. Completed hemisected gastrula from left side. Mesoderm, except at blastopore, is in enterocoelic fold and mostly invisible. (See text.) *H*. Young hemisected embryo from left side. Neural folds forming and covering blastopore to form neurenteric canal. Only one layer in "fold" at this stage. (See text.)

side. Because of the general form of the blastula this flattening wall or plate when viewed from the future posterior, has the shape of a triangle with slightly curved sides. The widest side of this triangle is antero-dorsal toward the larger end of the pear. The other two sides extend postero-ventrally until they meet at the smaller end. As will presently appear, the broad transverse antero-dorsal edge of the plate will constitute the dorsal lip of the blastopore. The other two edges will constitute the ventro-lateral lips, there being no strictly ventral lip unless one chooses to regard the angle where these last named lips meet as such. Hence the blastopore as it develops will, for a time at least, be triangular rather than round (Fig. 51, *E*).

The flattening of the hypoblastic plate is further accentuated, and presently the cells so affected begin to move inward somewhat as in the typical illustration of the invagination process.² In this case, however, the movement is not equal on all sides. Instead it is greatest at the broad transverse dorsal lip, becoming less as one goes posteriorly along the postero-lateral lips. It is somewhat as though the hypoblastic plate were a door swinging inward, with the more posterior part of the postero-ventral lips acting as the hinge. It is clear, however, that the swinging in movement cannot occur to the exclusion of other processes. If it did a break would necessarily take place between the plate and the part of the lip from which it is moving away. That such a break does not occur is apparently due in part to the involution or inflection of cells at these regions of the lips, particularly at the dorsal lip. This in turn is made possible by active cell division. It may also be noted in this connection that the cells of the hypoblastic plate whose inner ends are distinctly rounded have become more columnar in shape, while those of the epiblast have become less columnar and more cubical (Fig. 51, *A*). These and other changes in shape of gastrular cells have already been noted as being the probable immediate cause of the process of involution. Another feature to be mentioned at this point is the fate of approximately six transverse rows of cells just at, and immediately anterior to, the dorsal blastoporal lip. As involution proceeds three of these rows

² The terms epiblast and hypoblast are not used by Conklin in his description of the gastrulation of *Amphioxus*. This is probably because of the effort of this author to emphasize the fact that the materials for all three germ layers are distinguishable, as noted, from the very beginning. However, it seems best to be consistent in our use of these terms. Therefore, we shall apply the names ectoderm, endoderm and mesoderm to these layers only after they have actually been set aside as definitive cellular sheets. Previous to that we have referred to the materials concerned as "potentially" this or that. During gastrulation the primary inner and outer layers will be designated as usual as hypoblast and epiblast.



90 THE EARLY DEVELOPMENT OF AMPHIOXUS

are turned over the edge of the lip and into the growing archenteric roof. These turned-in rows thus become a part of the hypoblast, while the other three remain outside as part of the dorsal epiblast. The former cells will eventually be the source of the notochord, while the latter, i.e., those not involuted, will furnish material for the neural tube. This will be referred to again when the origin of these structures is described.

Epiboly. — This process is typically thought of in connection with large yolked eggs in which a layer or layers of cells overgrow a mass of yolk. There is of course no such mass in the case of *Amphioxus*. Nevertheless part of the gastrulation process here is essentially epibolic, the gastrular cavity taking the place of solid nutrient material. This epiboly is accomplished initially in the following manner: The ventro-lateral lips tend to become continuous and begin to grow dorsally while at the same time the dorsal lip becomes more arched. In this way the originally triangular blastopore loses its angles and becomes more or less of a transversely placed oval. The dorsal side of this oval now constitutes the dorso-lateral lip of the blastopore, and the ventral side the ventro-lateral lip. All parts of the oval then grow toward one another with the lateral parts moving relatively more rapidly than the dorsal and ventral. As a result of these activities the oval blastopore presently becomes a small circular opening. Thus an essentially epibolic process is responsible for covering over the gastrular cavity. At the same time there is also occurring a gradual lengthening of the entire gastrula owing to active cell division in the blastoporal lips and elsewhere. In this manner what might be described as a double walled tube-shaped sac is formed, the outer layer of the wall being epiblast, and the inner wall hypoblast (Fig. 51, *D, F, G, H*). Henceforth this sac-like structure may be referred to as a larva or embryo. Accompanying these movements there has necessarily been a redistribution of the material of the mesodermal crescent which according to Conklin lies in the two original ventro-lateral lips. The details of this rearrangement will be taken up in connection with the history of the definitive mesoderm.

Convergence. — According to previous accounts gastrulation in this animal has also involved a distinct process of concrescence or flowing together of the material from the two sides of the dorsal lip along a median line. Conklin, however, asserts very positively that nothing of this sort occurs. Nevertheless, he does admit that regions in and about the lip do contribute substances to the embryo by a process which is

essentially convergence as described below. In correlation with this view Conklin never refers to the lips as the germ ring.

During the above processes the ectoderm cells develop cilia which vibrate and thus cause the embryo to rotate slowly within the egg membrane.

THE CENTRAL NERVOUS SYSTEM

The early development of the nervous system occurs more or less simultaneously with the differentiation of the notochord and the mesodermal somites. It is convenient, however, to describe these three processes separately, and we shall therefore begin with the nervous system.

The Neural Plate and the Neural Folds.—As previously suggested there exist in the early gastrula about six rows of approximately ten to twelve cells, each extending transversely across the embryo just at, and anterior to, the dorsal lip of the blastopore. As indicated the row immediately adjacent to the lip, and the two next anterior rows, are presently involuted to the roof of the archenteron. Here they will be referred to later in connection with the development of the notochord. The three cell rows which remain outside, together with all the rest of the epiblast, may henceforth be called ectoderm. These three rows (Fig. 51, *E*) then give rise to the nervous system in the following manner: As the embryo increases in length the cells in these rows divide, along with the others, and so continue to extend from the margin of the dorsal blastoporal lip very nearly to the anterior end of the embryo. They thus constitute an elongated band of material about twelve cells in width. It is the *neural* or *medullary plate*. At the same time the ectoderm along each side of this plate becomes slightly elevated, and these elevations then begin to grow toward one another above the plate. As this process continues the ectoderm constituting the elevations becomes separated from that at the margins of the plate, and the former gradually approach each other until they meet and fuse along the median line (Fig. 52, *A, B, C*). Thus the medullary plate itself is entirely roofed over, and during the process it is customary to speak of the free edges of the two approaching layers of ectoderm as the *medullary* or *neural folds*. As a matter of fact, however, these layers obviously involve none of the actual medullary plate, and constitute only the outer half of a true fold (Fig. 52). Hence the neural folds, as here indicated, are but partly homologous with the similarly named structures in most higher forms (see below). It should now be added that the phenomena just described do not occur everywhere simultaneously. The depression of the neural

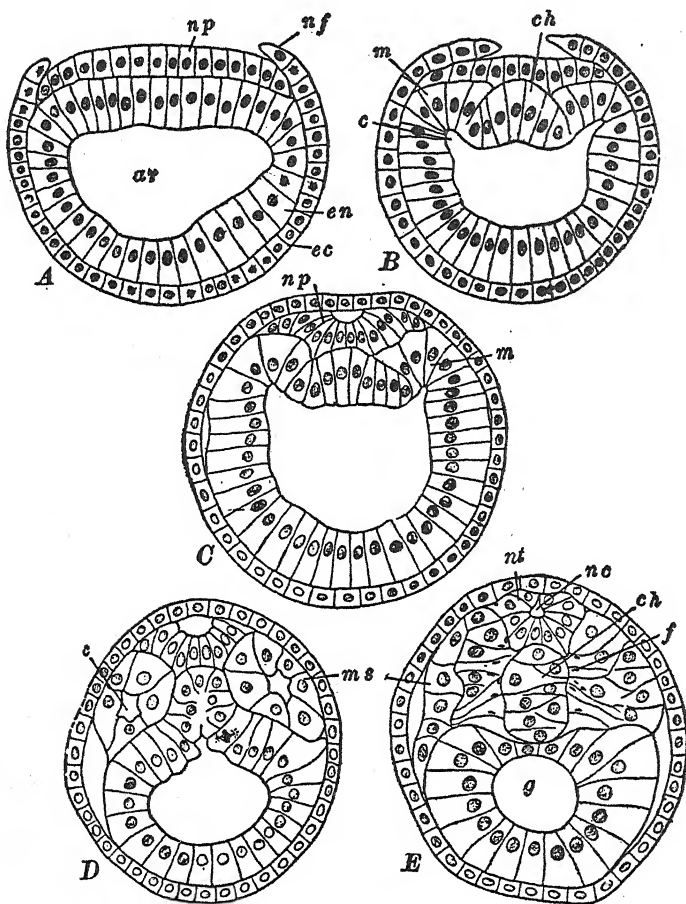


Fig. 52. — Transverse sections through young embryos of *Amphioxus*, showing formation of nerve cord, notochord and mesoderm. From Kellicott (*Chordate Development*). After Cerfontaine. A. Commencement of the growth of the superficial ectoderm (neural folds) above the neural or medullary plate. B. Continued growth of the ectoderm over the neural plate. Differentiation of the notochord, and first indications of the mesoderm and enterocoelic cavities. C. Section through middle of larva with two somites. Neural plate folding into a tube. D. Section through first pair of mesodermal somites, now completely constricted off. E. Section through middle of larva with nine pairs of somites. Neural plate folded into a tube. Notochord completely separated. In the inner cells of the somites, muscle fibrillae are forming.

c. Enterocoel. ch. Notochord. ec. Ectoderm. en. Endoderm. f. Muscle fibrillae. g. Gut cavity. m. Unsegmented mesoderm fold. ms. Mesodermal somite. nc. Neurocoel. nf. Neural fold. np. Neural plate. nt. Neural tube.

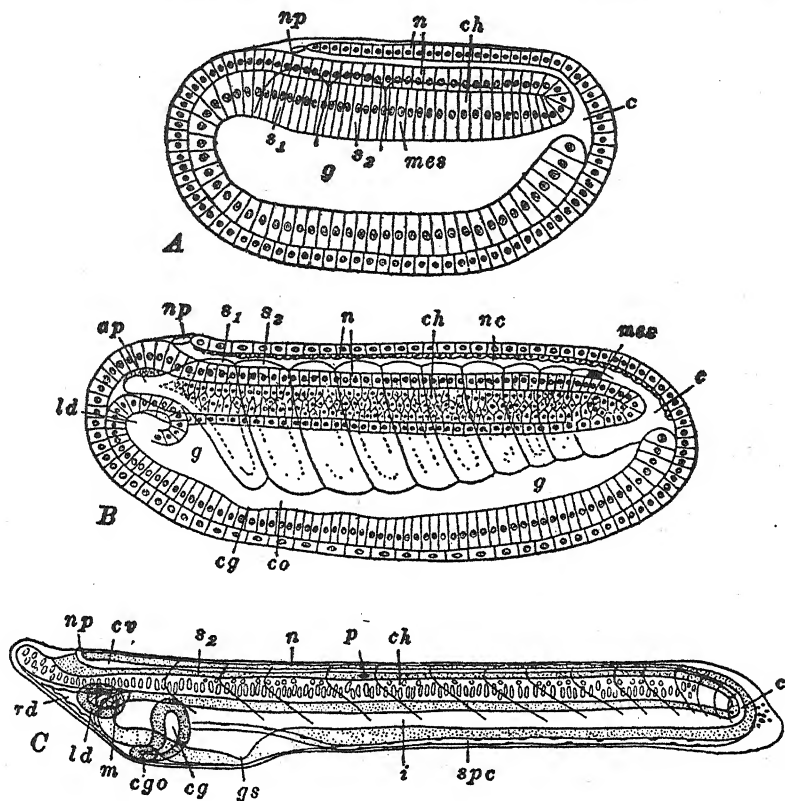


Fig. 53.—Optical sections of young embryos of *Amphioxus*. From Kellicott (*Chordate Development*). After Hatschek. The cilia are omitted. *A*. Two-somite stage, approximately at the time of hatching, showing relation of neuropore and neurenteric canal. *B*. Nine-somite stage, showing origin of anterior gut diverticula. *C*. Fifteen-somite stage. End of the embryonic period.

ap. Anterior process of first somite. According to Conklin the existence of this is doubtful. *c*. Neurenteric canal. *ch*. Notochord (or its rudiment, in *A*). *cg*. Club-shaped gland (or its rudiment in *B*). *cgo*. External opening of club-shaped gland. *co*. Coelomic cavity of somite. *cv*. Cerebral vesicle. *g*. Gut cavity (enteron, mesenteron). *gs*. Rudiment of first gill slit. *i*. Intestine. *ld*. Left anterior gut diverticulum (preoral pit in *C*). *m*. Mouth. *mes*. Unsegmented mesoderm. *n*. Nerve cord (or its rudiment, in *A*). *nc*. Neurocoel. *np*. Neuropore. *p*. Pigment spot in nerve cord. *rd*. Right anterior gut diverticulum (preoral head cavity in *C*). *s1 s2*. First and second mesodermal somites. *spc*. Splanchnocoel (body cavity).

plate begins just in front of the blastopore, and extends anteriorly, while the fusion of the neural folds begins slightly further forward and extends both ways. The latter process is further augmented, according to Conklin, by the continued upgrowth of the ventral lip of the blastopore over the dorsal side. Insofar as this occurs the layer so arising simply fuses with that of the lateral neural folds as described above (Fig. 51, *H*). As a result of these processes the blastopore is presently entirely roofed over.

The Neuropore. — Although the blastopore has been covered in the manner just indicated, the archenteron still communicates with the exterior. This is accomplished by means of the space extending along the back of the embryo between the neural folds above and the medullary plate beneath. This space leads from the blastopore forward to the point where the folds are still in the process of uniting, and here opens to the outside. This opening is termed the *neuropore*, and is constantly advancing as the meeting of the folds continues. At the time of hatching, which occurs eight to fifteen hours after fertilization, this point is generally somewhat anterior to the middle of the embryo (Fig. 53).

The Neurocoel and the Neurenteric Canal. — When in approximately this condition as regards the nervous system, the young embryo breaks out of the egg membranes. Further development of this system then proceeds as follows. The process of roofing over the medullary plate is completed so that the neuropore is carried almost to the anterior end of the animal. The center of the neural plate is then somewhat further depressed, while its edges³ are bent upward and inward until they meet (Fig. 52, *C, D, E*). There is thus formed within the old space between the archenteric roof and the fused neural folds, a new tube — the *neural tube*, containing a canal, the *neural canal* or *neurocoel* (Fig. 53, *B*). The inner surface of this canal is evidently that of the original neural plate, and hence as might be expected, is lined with cilia. From the method of its formation also, it is clear that anteriorly the neurocoel will open to the exterior at the neuropore and that posteriorly it will still communicate with the archenteron through the blastopore. This posterior passageway through the blastopore into the neurocoel is now termed the *neurenteric canal*. Both neurenteric canal and neuropore remain open throughout the embryonic period, i.e., until the mouth is formed.

Later, the anterior portion of the neural tube widens somewhat to

³ These edges are mostly homologous with the inner or nervous portion of the neural folds as described in the Frog (see below).

form the rudiment of a brain while within the tube at this and other points, pigment spots appear. These, or the tissues externally adjacent to them, are probably light receptors.

This is as far as it is necessary to consider the development of the nervous system in *Amphioxus*. In comparing this development with that of most higher Chordates there will be found a fundamental similarity. There is one variation in detail, however, which, though it has already been indicated, deserves a further word of emphasis. In all those cases where the neural tube is formed by so-called neural folds it is only in *Amphioxus* that the completion of the real tube occurs later than, and hence separately from, the overgrowth and fusion of the folds. Indeed, as will appear from reference to Fig. 43, in all true Vertebrates in which the tube arises by fold formation, the edge of the plate remains united to the edge of the outer layer of overgrowing ectoderm until the folds from opposite sides meet. Thus in these latter cases the structures so named are truly folds, instead of being only the outer half of a fold as in *Amphioxus*.⁴

THE DEVELOPMENT OF THE NOTOCHORD, MESODERMAL SOMITES AND COELOM

The Notochord. — It will be recalled that in connection with the development of the nervous system reference was made to the occurrence in the early gastrula of three transverse rows of cells immediately adjacent to the dorsal lip of the blastopore. It was indicated that these cells are involuted into the roof of the archenteron. As the gastrula increases in length the hypoblast cells of these inturned rows multiply along with the outer epiblastic cells which are to give rise to the neural plate. Thus like those of the latter structure they produce a lengthening band ten or twelve cells wide which forms the archenteric roof. As in the case of the neural plate, this band then begins to fold, but in this instance the edges are directed downward instead of upward. Also as the sides of the fold come together the cells tend to interdigitate (Fig. 52, *B, C, D, E*). In this manner a solid rod of tissue is formed, the *notochord*, lying immediately beneath the neural tube. Although at first the notochordal cells are wedge shaped and interdigitated, they eventually become disc-shaped and in a cross sectional view appear, as Conk-

⁴ The peculiar method by which the neural tube is formed in *Amphioxus* must probably be regarded as specialized rather than primitive. Upon this same basis some authorities do not homologize the overgrowing ectoderm with any part of a true neural fold.

lin says, to be piled like a stack of coins. Finally their nuclei and protoplasm disappear, leaving a clear substance, presumably possessing a turgor which helps give rigidity to the entire structure. Posteriorly the notochord ceases at the neurenteric canal, while anteriorly it eventually reaches to the extreme anterior end of the embryo in front of the brain (Fig. 53, C). In this last respect *Amphioxus* differs from other Chordates in which the notochord always stops beneath the mid-brain.

The Mesodermal Somites and Coelom. — It will be recalled that according to Conklin the material destined to be mesoderm, like that destined to form ectoderm and endoderm, is differentiated and visible clear back in the fertilized egg. Here the potential mesodermal substance is gathered in the form of a crescent across what will presently be the posterior side of the larva. As segmentation occurs this crescent, as was noted, retains its position, and thus in the early gastrula comes to lie just inside the ventro-lateral lips of the triangular blastopore. Its middle section is at the median and ventral-most region where the two lips may be said to meet one another, while the two horns of the crescent extend antero-dorsally to the angles made by the junction of the ventro-lateral lips with the dorsal lip (Fig. 51, E, F). It will now be recalled that the two ventro-lateral lips presently become one, the angle between them never having been a very acute one. Thus as previously noted the entire blastopore takes on the shape of a transversely placed oval, the lower lip of which becomes identical with the posterior border of the crescent. As already indicated, as this ventral lip then moves upward, the middle part of the crescent is likewise raised, and the sides or horns assume an almost horizontal antero-posterior position. Meanwhile the cells of this potential mesodermal region have become the most actively dividing in the embryo, and hence the smallest. With the ensuing drawing together of the blastoporal lips and the lengthening of the embryo, the material in the former mesodermal crescent suffers a further redistribution as follows: The posterior part of this potential mesodermal material, i.e., the part which has formed the middle of the crescent, now passes around the ventral and lateral side of the contracted blastopore just within its margin. As a result of the lengthening process, the former horns then proceed forward in two bands, each of which is six to nine cells in width. Each band is immediately adjacent to the edges of the rather flat archenteric roof which is about to fold downward in the manner indicated to form the notochord. Thus the hypoblastic bands of potential mesoderm occupy the angles uniting the roof of the archenteron with its sides. Before-proceeding further with

the fate of these bands it is necessary to pause a moment to consider one or two theoretical matters.

It will be recalled that under the general discussion of the processes of gastrulation in the preceding chapter it was indicated that the lip of the blastopore is sometimes referred to as the germ ring. This is done, it was said, on the ground that this lip or ring comprises the "germ" of the embryo in that each side of it contains half of the embryonic anlage which is then brought into contact with the other half by concrescence of the blastoporal lips to form a whole. It was suggested, however, that this is scarcely true in the sense originally conceived, and the present case affords a good instance of the ways in which the original conception has had to be modified. First, it is quite evident that only in the vaguest sense can a half embryonic anlage be said to lie in the lateral blastoporal lips. All that can be said is that certain materials for the embryo do pass into it from within or near the lips of the blastopore, the potential mesoderm from the ventro-lateral lips, and potential neural and notochordal material from the dorsal lip. Secondly, as we have seen, these materials do not assume their definitive positions by a simple process of the concrescence of two sides, though the process may be thought of as a kind of convergence or confluence. If the term germ ring is to continue to be employed at all therefore it can only be with a considerably modified significance as indicated in this instance.

Returning now to the further history of the potential mesoderm it soon appears that the hypoblastic bands on either side of the notochordal region very shortly become folded so as to form grooves with the grooved side of the fold facing the archenteron (Fig. 52, B, C). In this manner this part of the hypoblast becomes cut off from the archenteron, and thus becomes definitive mesoderm. At the same time the hypoblast to the lateral side of each groove is drawn toward the midline. Here, as the notochord is also becoming folded off, it is finally drawn completely together so as entirely to line the archenteric cavity as definitive endoderm. In both these situations it may be noted that the folding process is accompanied by, and probably dependent upon, a change in the shape of cells, causing them to roll over a lip. The general significance and widespread occurrence of this mechanism for cell rearrangement was pointed out in connection with the general discussion of involution as a method of gastrulation.

Meanwhile as the folding process is taking place the mesoderm forming each lateral groove is becoming distinctly moniliform, i.e., transverse constrictions are developing in it particularly at the anterior end.

In this way there are soon produced anteriorly definitely separate mesodermal blocks, each with a small cavity within it. These blocks are termed *mesodermal somites*, and it is to be noted that they are formed essentially as *enterocoelic pouches* by a process of folding off from the archenteron in the presumably primitive manner. Only the first two or three somites thus formed, however, have actual cavities at this time. Posteriorly the groove closes as it forms, and the cavities within the constricted blocks of mesoderm form later. Whenever formed such cavities represent the beginnings of the *coelom*, and certain other spaces to be described presently. Eventually as many as sixty-one pairs of somites are thus produced. In this connection it must be clearly noted that the term somite as used with respect to *Amphioxus* applies both to the myotomal region (segmental plate in true Vertebrates), and to the lateral plate, instead of only to the former. This will become apparent from what follows.

Before proceeding with the further development of the somites a word should be said concerning a certain classification of mesoderm which is sometimes made on the basis of the method of its setting aside as such. Thus it has been seen that the mesoderm of the first eight or ten somites arises by the folding off of material which just preceding this process lies within the archenteric wall. Later somites, however, arise more directly from material which is paid into the dorso-lateral regions from the lips of the blastopore as the embryo elongates. Hence the somite material (mesoderm) which is set aside in the former manner has been called *gastral*, while the latter arising more directly from the lips of the blastopore is called *peristomial*. In view of the fact, however, as brought out by Conklin, that apparently all the mesoderm has its origin from material at first lying within the blastoporal lips, such a distinction as the above largely breaks down. All of it is really peristomial.

THE FURTHER DEVELOPMENT OF SOMITES AND COELOM

By the time seven or eight pairs of somites have been formed, it becomes evident that only the members of the first pair and the upper parts of the second are exactly opposite one another. Posterior to this the somites of the left side are more and more in advance of their mates on the right, until soon they alternate. This is a feature peculiarly characteristic of *Amphioxus*.

The Lateral Plate.—At the stage of fourteen or fifteen somites certain further changes begin to appear in the more anterior pairs. In each somite the enterocoel becomes larger, while the walls of the ven-

tral portion below the level of the notochord become thinner. At the same time this portion begins to lengthen in a postero-ventral direction, the region thus affected being known as the *lateral plate*.

The outer wall of this plate next to the ectoderm is called the *somatic* or *parietal* mesoderm, while the inner wall next to the enteron is *splanchnic* or *visceral* mesoderm. The part of the enterocoel which lies between them is the *splanchnocoel* or true coelom. The lateral plates on each side of the embryo continue to grow ventrally until they finally meet. Presently the ventro-median wall which at first separates the splanchnocoels of the two sides largely disappears, as well as the walls separating the successive splanchnocoels of the same side. Thus the splanchnocoel or coelom becomes completely continuous throughout the entire lateral and ventral region of the animal.

The Myotomal Region.—While this is going on in the lower portion

of each somite, the upper portion on a level with the notochord is assuming the < shape characteristic of the adult. It is also becoming thicker, largely as a result of the horizontal flattening of its cells in the wall adjacent to the notochord. These cells presently become differentiated as muscle cells, extend throughout the length of the somite, and nearly obliterate the enterocoel in this upper region. The thickened muscular tissue of each somite is then called a *myotome*, while the slight enterocoelic space still remaining between the latter and the outer unthickened

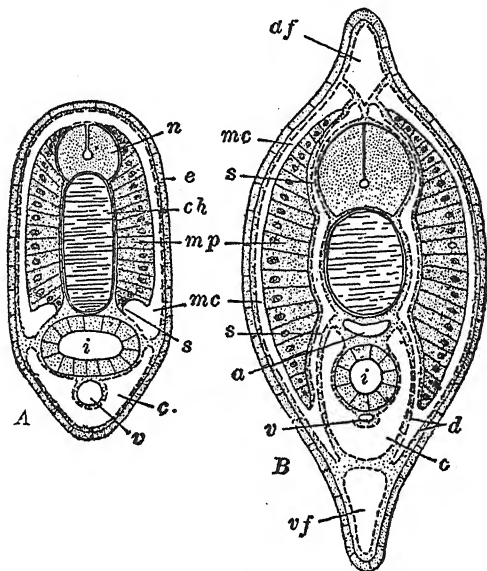
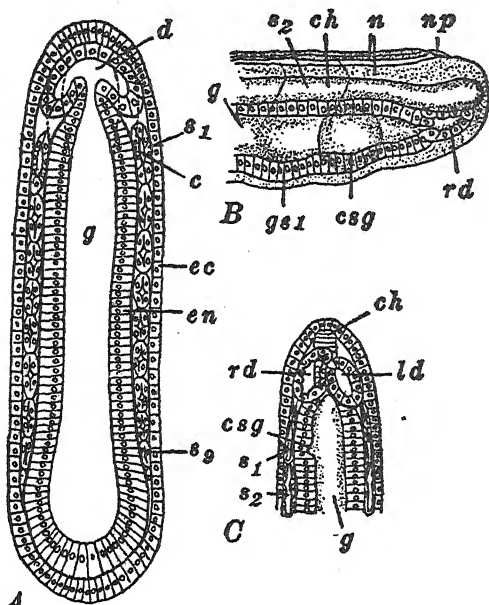


Fig. 54. — Diagrams of transverse sections through *Amphioxus* larvae. From Kellicott (*Chordate Development*). A. Through the body region of a larva with five gill slits, showing separation of mycoel and splanchnocoel (coelom). B. Through the region between atriopore and anus of young individual, shortly after metamorphosis, showing relations of sclerotome. After Hatschek. a. Dorsal aorta. c. Coelom (splanchnocoel). ch. Notochord. d. Dermatome. df. Dorsal fin cavity. e. Epidermis. i. Intestine. mc. Mycoel. mp. Muscle plate (myotome). n. Nerve cord. s. Sclerotome. v. Subintestinal vein. vf. Ventral fin cavity.



A

Fig. 55.—Sections through young *Amphioxus* embryos showing the origin of the anterior gut diverticula. From Kellicott (*Chordate Development*). After Hatschek. The cilia are omitted. A. Frontal section through embryo with nine pairs of somites. (See Fig. 53, B.) The dotted line marks the course of the gut wall ventral to the level of the section. B. Optical sagittal section through anterior end of embryo with thirteen pairs of somites, showing position of right anterior gut diverticulum. C. Same in ventral view.

c. Coelomic cavity of somite. ch. Notochord. csq. Rudiment of club-shaped gland. d. Rudiment of anterior gut diverticula. ec. Ectoderm. en. Endoderm. g. Gut cavity (enteron, mesenteron). gsl. Rudiment of first gill slit. ld. Left anterior gut diverticulum. n. Nerve cord. np. Neuropore. rd. Right anterior gut diverticulum. s₁s₂s₉. First, second and ninth mesodermal somites.

wall is termed a *myocoel* (Fig. 54). Later, between the myotome and the lateral plate there develops a horizontal partition which acts as a boundary between the two regions. Eventually also there grows out from the ventral region of the myotomal portion of each somite a fold of tissue which presently becomes divided into two parts. One part then extends upward between the myotome and the notochord and nerve cord as the *sclerotome*. The inner layer of this sclerotomal part finally forms the *skel-etogenous sheath* for the latter structures, while its outer layer forms the covering or *fasciae* for the inner sides of the myotomes themselves; the latter have no fasciae on their outer sides, as they do in the Craniates. The

other portion of the original fold meanwhile extends outward and downward between the somatic layer of the lateral plate and the ectoderm. This fold, together with the outer unthickened wall of the upper or myotomal region, is known as the *dermatome*. The upper myotomal portion of the dermatome gives rise to the *cutis layer* of the integument in the dorsal part of the animal, while the fused inner and outer sheets of the dermatomal fold constitute the same layer ventrally. These points should

be kept in mind, in connection with the development of homologous parts in the higher Vertebrates.

The Anterior Gut Diverticula. — Although it is not strictly connected with the formation of the somites, we may mention in closing the appearance of certain diverticula of the archenteron, which in their early stages are not unlike enterocoels.⁵ When about seven pairs of somites have been formed, there develops from the dorsal wall of the gut in front of the most anterior somites a transverse ridge. This ridge thus produces a sort of dorsal bay or pouch at the anterior extremity of the gut beneath the notochord (Fig. 53, *B*). The sides of this bay then push upward on either side of the notochord, thus forming two dorso-lateral pouches. The ventral edge of the transverse ridge now grows anteriorly cutting off these two pouches ventrally from the anterior extremity of the gut beneath them. Each then develops in its own peculiar fashion (Figs. 53, 55). The right one becomes greatly enlarged, assumes a median position, and occupies the whole of the space beneath the chorda and in front of the enteron. The left remains smaller and finally acquires an opening to the outside of the head known as the *pre-oral pit* (Fig. 53, *C*).

The later development of *Amphioxus* is too highly specialized to help us much in an understanding of the higher and more typical Chordates. It will therefore be omitted. Those students who are interested in the further history of this animal, however, will find a good brief account with references to original papers in Kellicott's *Chordate Development*. They should also note the references at the conclusion of this chapter.

REFERENCES TO LITERATURE

- Cerfontaine, P., "Recherches sur le développement de l'*Amphioxus*," *Arch. Biol.*, XXII, 1906.
 Conklin, E. G., "The Embryology of *Amphioxus*," *Jour. Morph.*, LIV, 1932. — "The Development of Isolated and Partially Separated Blastomeres of *Amphioxus*," *Jour. Exp. Zool.*, LXIV, 1933.
 Garbowski, T., "Amphioxus als Grundlage der Mesodermtheorie," *Anat. Anz.*, XIV, 1898.
 Hatschek, B., "Studien über Entwicklung des *Amphioxus*," *Arbeit. zool. Inst. Wien.* IV, 1882. "Ueber den Schichtenbau von *Amphioxus*" (Verhand. d. Anat. Gesell., II), *Anat. Anz.*, III, 1888.
 Klaatsch, H., "Bemerkung über die Gastrula des *Amphioxus*," *Morph. Jahrb.*, XXV, 1897.
 Kowalewsky, A., "Entwicklungsgeschichte des *Amphioxus lanceolatus*," *Mém. Acad. Impér. St. P.*, VII, 11, 1867. — "Weitere Studien über die Entwicke-

⁵ By some authorities (Hatschek, MacBride) these structures are regarded as actual, though modified, mesodermal somites.

102 THE EARLY DEVELOPMENT OF AMPHIOXUS

- lungsgeschichte des *Amphioxus lanceolatus*, nebst einem Beitrage zur Homologie des Nervensystems der Würmer und Wirbelthiere," *Arch. mikr. Anat.*, XIII, 1877.
- Legros, R., "Sur quelques cas d'asyntaxie blastoporale chez l'*Amphioxus*," *Mitt. Zool. Stat. Neapel*, XVIII, 1907. — "Sur le développement des fentes branchiales et des canicules de Weiss-Boveri Chez l'*Amphioxus*," *Anat. Anz.*, XXXIV, 1909. — (Published anonymously.) "Sur quelques points de l'anatomie et du développement de l'*Amphioxus*: Notes préliminaires. 1. Sur le néphridium de Hatschek," *Anat. Anz.*, XXXV, 1910.
- Lwoff, B., "Ueber einige wichtige Punkte in der Entwicklung des *Amphioxus*," *Biol. Centr.*, XII, 1892. — "Die Bildung der primären Keimblätter und die Entstehung der Chorda und des Mesoderms bei den Wirbelthieren," *Bull. Soc. Impér. Moscou*, II, 8, 1894.
- MacBride, E. W., "The Early Development of *Amphioxus*," *Q. J. M. S.*, XL, 1898. — "Further Remarks on the Development of *Amphioxus*," *Q. J. M. S.*, XLIII, 1900. — "The Formation of the Layers in *Amphioxus* and its Bearing on the Interpretation of the Early Ontogenetic Processes in Other Vertebrates," *Q. J. M. S.*, LIV, 1909.
- Morgan, T. H. and Hazen, A. P., "The Gastrulation of *Amphioxus*," *Jour. Morph.*, XVI, 1900.
- Samassa, P., "Studien über den Einfluss des Dotters auf die Gastrulation und die Bildung der primären Keimblätter de Wirbelthiere, IV. *Amphioxus*," *Arch. Entw.-mech.*, VII, 1898.
- Sobotta, J., "Die Reifung und Befruchtung des Eies von *Amphioxus lanceolatus*," *Arch. mikr. Anat.*, L, 1897.
- Wiley, A., "The Later Larval Development of *Amphioxus*," *Q. J. M. S.*, XXXII, 1891. — *Amphioxus and the Ancestry of the Vertebrates* (Columbia University Biological Series II), New York, 1894.
- Wilson, E. B., "Amphioxus and the Mosaic Theory of Development," *Jour. Morph.*, VIII, 1893.

PART II

THE DEVELOPMENT OF THE FROG



THE FROG: FROM THE PRODUCTION OF THE GERM CELLS THROUGH GASTRULATION

THE embryology of the Frog, *Rana sp.*, will be taken up as the first example of the development of a true Vertebrate, being a valuable object for such study for the following reasons: In the first place its earlier history furnishes an excellent transition between the corresponding stages in Amphioxus and those in animals which are more highly evolved. Second, the later development of the Frog is also very suggestive from an evolutionary point of view. Thus it illustrates in a striking manner the transformation of a purely aquatic gill-breathing Vertebrate into one which breathes largely by lungs, and is capable of extended existence on land. Third, in the course of its development the Frog shows the origin of practically all of the fundamental Vertebrate systems. Yet in many cases these systems remain in a rather primitive condition, and are thus helpful to an understanding of the complications which are met with in other types. Fourth, the development of the Frog is important both because of the thoroughness with which it has been observed under normal conditions, and also because of the active experimental work which has been and is being done upon it and its near relatives. Lastly, there are also certain practical considerations. The living material is usually available at an appropriate time of year, it is easy to handle, and the young can be readily cared for under laboratory conditions.

THE REPRODUCTIVE ORGANS OF THE ADULT, OÖGENESIS, AND THE EXTRUSION OF THE OVA

THE MALE ORGANS

The Testes. — There are two testes in the Frog, each one lying in the dorsal region of the coelom, close to the kidney (Fig. 56). Each is enveloped by the peritoneal epithelium, which is fused above the organ into a two-layered sheet of tissue, like a mesentery. This sheet attaches the testis to the body wall and is termed the *mesorchium*. In appearance

each testis is a white ovoid body which may be a half inch or so in length. In some species in which the sperm are produced continuously, the size of the organ remains fairly constant. In others, however, in which spermatogenesis is chiefly confined to the breeding seasons, the dimensions vary considerably. This variation is nevertheless relatively small compared to what always occurs in the ovary.

In structure each testis consists essentially of a mass of *seminiferous tubules*. These are grouped into lobules and the latter again into lobes separated by thin partitions of supporting or connective tissue. This tissue also covers the whole organ in a coat called the *tunica albuginea*, outside of which is finally the peritoneum. The walls of the tubules are lined internally with follicle or nutrient cells (*Sertoli cells*), while between the latter and the lumen of each tubule come groups of germ cells in various stages of development, those in any given group being in approximately the same stage. As the cells of a group reach the condition of spermatids their heads are gathered together and the tips embedded in a Sertoli cell. Finally when fully ripe the spermatozoa are liberated into the tubular lumen.

To the anterior end of each testis is attached a *fat body*, composed of a mass of yellow streamers. Its function is uncertain. Inasmuch as the animals do not eat during the breeding season, however, it may serve as an extra supply of nutrient material to be drawn on at this time.

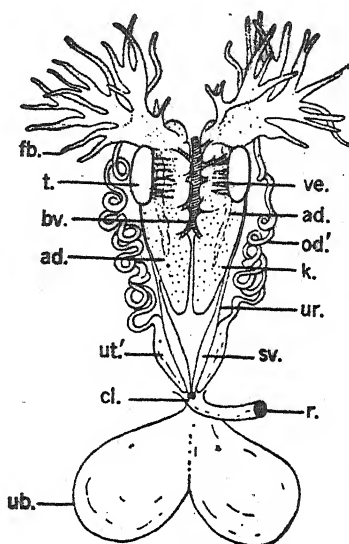


Fig. 56. — The male urinogenital system of the adult Frog (*Rana pipiens*) viewed from the ventral side. The testes in this case are medium sized. The urinary bladder and rectum have been dissected out and reflected posteriorly. Otherwise in the ventral view they would cover the lower part of the reproductive organs. Note the large fat bodies as compared with those in the female. Also note the rudimentary oviducts. In many species of Frogs these ducts do not develop so far in the male as in *R. pipiens*. They have no known function in this sex.

ad. Adrenals. bv. Blood vessel. cl. Cloaca. fb. Fat bodies. k. Kidney (mesonephros). od'. Rudimentary oviduct. r. Rectum. sv. Seminal vesicle. t. Testis. ub. Urinary bladder. ur. Ureter, in the male serving also as a vas deferens. ut'. Rudimentary uterus. ve. Vasa efferentia.

The Sperm Ducts.—The tubules of each testis open into about a dozen fine ducts, the *vasa efferentia*. These connect with some of the more anterior kidney tubules, which thus function as continuations of the *vasa efferentia* as well as in excretion. These tubules in turn of

course empty into each kidney duct, which therefore acts as both *ureter* and sperm duct (*vas deferens*). The two *vasa deferentia* are dilated just before entering the cloaca to form the *seminal vesicles*. In these, the sperm are stored previous to discharge.

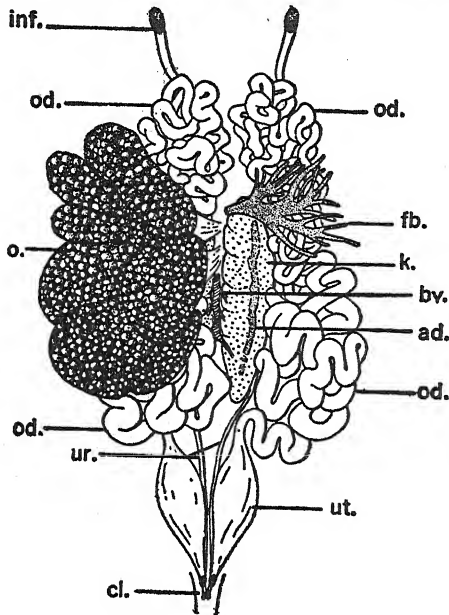


Fig. 57.—The female urinogenital system of the adult Frog (*Rana pipiens*) viewed from the ventral side. The left ovary has been removed, showing the fat body, kidney and oviduct upon that side. The right ovary full of nearly mature eggs remains in place. Note that the fat body is smaller than in the male, having presumably suffered depletion during the development of the eggs. The urinary bladder and rectum are omitted from the figure, but occur in the same position as in the male.

inf. Infundibulum. *o.* Ovary. *od.* Oviduct. *ut.* Uterus. Other abbreviations as in Fig. 56.

THE FEMALE ORGANS

The Ovaries.—The ovaries are also paired organs and occupy the same relative position as the testes (Fig. 57). As in the case of the latter, each is suspended from the body wall by a double sheet of peritoneal tissue in this instance called the *mesovarium*. Unlike the testes, however, the ovaries always vary greatly in size and appearance, depending upon the time of year. After ovulation in the spring they

appear as flattened cream colored organs, about three-quarters of an inch long in *Rana pipiens*, with a few dark specks scattered through them. As the *oögonia* for the ensuing season multiply and presently grow into *oöcytes*, however, the organs increase immensely in size, and by the end of the summer they occupy a large share of the body cavity. They are now lobulated in form, and exhibit a characteristic black and white speckling, due to the color of the ripe eggs. Under normal circumstances they

remain in this condition throughout the winter. As will be indicated later, however, the eggs are completely developed, and by artificial means such ovaries can be made to ovulate viable ova at any time.

In structure, the ovary consists of a number of compartments, whose outer walls are formed of connective tissue or *stroma*. Within the compartments the oögonia may be in the process of multiplication, as suggested above, or if this stage has passed the compartments will be filled with oöcytes. Each of these oöcytes is surrounded by a single layer of flattened cells which constitute its *follicle*. Outside of this is another layer termed the *theca*, which serves to attach the ovum to the wall of its compartment. This theca in turn is divided into an outer layer containing chiefly blood vessels, the *theca externa*, and an inner layer of smooth muscle fibers, the *theca interna*.

Attached to the anterior end of each ovary is a fat body similar in appearance, and presumably in general function, to those connected with the testes.

The Oviducts. — These are long convoluted tubes whose size and convolutions are somewhat increased during the breeding season. They open anteriorly into the coelom by a ciliated funnel, the *infundibulum*. Posteriorly they open into the cloaca. Throughout the greater part of their length the walls are quite thick, especially during breeding time. This thickening is due to the hyper-development of numerous simple tubular glands which secrete the gelatinous covering of the eggs. The lumen of the ducts is lined by ciliated epithelium. At the posterior end, each duct widens and its walls become thinner and very elastic. These dilated regions, known as the *uteri*, serve for storing the ova just prior to extrusion. Each duct is covered by a layer of peritoneum and slung from the dorsal body wall in the same manner as are the gonads.

OÖGENESIS

The Oögonia. — The normal breeding season, as already suggested, occurs in the spring or early summer. At this time the ovaries are emptied of ripe eggs, and the relatively few oögonia which remain begin to multiply to produce the eggs for the next season. These occur in nests, and in each such nest only one cell is destined finally to become an ovum, the others constituting its follicle. As soon as an ovum has become definitely differentiated as such, and its follicle formed, the period of growth and membrane formation sets in.

The Growth Period. — When this period has been reached the young ovum or oöcyte, as it may now be called, begins to accumulate

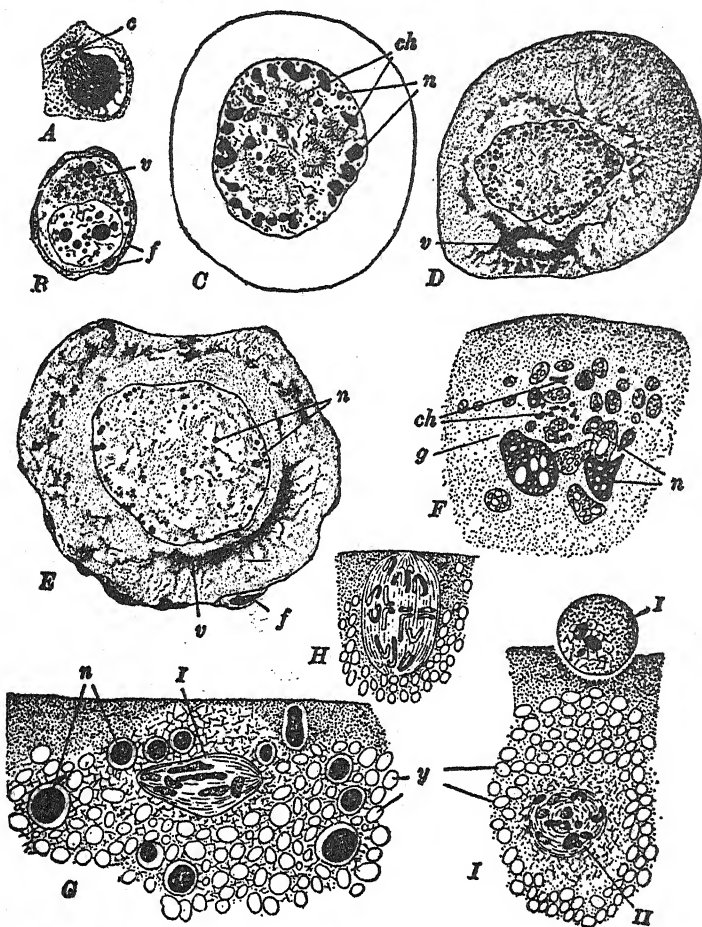


Fig. 58.—Oogenesis in the Frog (*R. temporaria*). From Kellicott (*Chordate Development*). A–E, after Lams. F–I, after Lebrun. A. Primary oocyte in synizesis. B. Primary oocyte with vitelline substance (yolk) of mitochondrial (chromidial?) origin in the cytoplasm. C. Primary oocyte showing feathery chromosomes and chromatin nucleoli. D. Primary oocyte with ring-like vitelline mass. E. Primary oocyte showing cytoplasm in two zones. F. Nuclear region of primary oocyte after dissolution of the nuclear membrane showing the small chromosomes and large chromatin nucleoli. Egg still in ovary. G. First polar spindle in primary position. From egg in body cavity. H. First polar spindle in metaphase. From egg in uterus. I. First polar body formed and second polar spindle forming. From eggs in uterus.

c. Centrosome. ch. Chromosomes. f. Follicle cells. g. Contents of germinal vesicle. n. Chromatin nucleoli. v. Vitelline substance of mitochondrial or Golgi body origin. y. Yolk plates. I. First polar spindle (polar body in I). II. Second polar spindle.

yolk. Before this starts nucleoli appear under the nuclear membrane. Also basophilic yolk-nuclei arise within the cytoplasm, and move first to the cell periphery and second to the nuclear periphery. The yolk then develops as granules just beneath the surface of the oöcyte. Though the source of these granules is uncertain, they may be derived from Golgi apparatus, the ground substance of the cytoplasm and the nucleus (Hibbard, '28). This layer of granules gradually widens, and the granules or platelets increase in size (Fig. 58, B, D, E). Eventually, the entire cytoplasm is filled with yolk (Kemp, '53), but the platelets are larger and more concentrated in what proves to be the vegetal half of the egg, thus making the latter telolecithal. What causes this polarity is still unknown. However, it is initiated very early by establishment of a ribonucleoprotein gradient (Brachet, '47b), a movement of the nucleus toward the animal pole, and by the collection of pigment beneath the surface of the animal hemisphere (Wittek, '52). This pigment soon spreads somewhat below the egg equator, shading in the vegetal hemisphere into a creamy white, thus giving the Frog ovary its speckled appearance. The ovum has meantime been acquiring two membranes. The inner membrane is an extremely delicate and close-fitting envelope secreted by the egg itself. It is therefore a true *vitelline* membrane, but is so thin that its actual existence is denied by some investigators. The outer covering is thin, but tough, and is formed by the follicle. Hence it is a secondary membrane or *chorion*.

While the ovum has been growing and acquiring its membranes, the nucleus has been passing through the stages preliminary to the first maturation division. In the female Frog these stages vary somewhat from what has been described as typical. The chief difference consists in the fact that after synizesis (Fig. 58, A), the chromatin threads are less visible so that when the heterochromosomes for the first maturation division later appear they seem to come from the chromatin nucleoli, but this is unlikely. They probably arise as usual from chromonema threads. (Fig. 58, C, D, E, F).

Before these chromosomes actually form, however, certain other events occur, as follows: The nucleus moves quite close to the animal pole, and the latter becomes slightly flattened. It is also claimed by some that the pigment of this pole withdraws to a certain extent just above the nucleus to form a small light area termed the *fovea*. The writer has never observed this in normal freshly spawned eggs, but this does not preclude its existence in eggs at the proper stage within the ovary or oviduct. Porter ('39) notes the existence of a small white spot at the

animal pole, with a dark dot within it marking the location of the second maturation spindle. This, however, was in eggs outside the ovary, and he makes no reference to the term "fovea." Likewise Rugh and others have noted that a fading of pigment occurs at the animal pole of aging eggs, but this again is in eggs outside the ovary, and probably not in a normal condition. The fovea as originally described therefore is, if it exists, apparently a separate phenomenon. The egg has now reached a diameter of from 1.5 to 3 mm., depending upon the species of Frog, and is ready for ovulation.

As noted, the series of processes leading to this result have taken place during the summer, and are virtually completed before the time of hibernating arrives. The eggs then normally remain in this condition until the period of spawning in the following spring.

OVULATION TO FERTILIZATION

Ovulation. — When spring arrives the ova are released from the ovary by the process known as *ovulation*. It was originally thought that the embrace of the male Frog known as *amplexus*, which occurs throughout spawning, was a necessary stimulus for the ovulatory process. As Rugh ('37) has so ably shown, however, *amplexus* really has nothing to do with it. This investigator clearly demonstrated that ovulation is brought about by an increase in the secretion of one of the pituitary hormones. Thus by injecting a sufficient number of minced pituitary glands into the body of a female Frog, ovulation can be artificially produced at any time when the ovary contains ripe eggs. Pituitaries from female frogs are more effective than those from males. However, any pituitary will probably do if properly prepared. The production of ovulation by this technique has been a great boon to Frog embryologists, since it is now possible to obtain fertilizable eggs at least nine months out of the year. The process of ovulation itself may be described as follows: The ovarian follicle breaks, and the ripe ovum is forced out through the epithelial covering of the ovary into the coelom. No matter in what region of the body cavity this act may occur, ciliary action on the peritoneum serves to convey the egg to the mouth or infundibulum of the oviduct. This is also ciliated and the ovum is drawn into the duct.

The First Maturation Division. — Before following the progress of the egg further it will be necessary to return for a moment to processes occurring within it.

At about the time of ovulation the nuclear membrane dissolves, and

shortly afterward the chromosomes of the first maturation figure arise from the nucleoli, as indicated above. As this figure forms, another peculiarity of maturation in the female Frog becomes evident, for neither centrioles, centrosomes nor asters are visible. Out of the fibrillar protoplasm, however, a spindle develops, division of the chromosomes occurs, and the first polar body is pinched off while the egg is in the upper part of the oviduct. This body lies just beneath the chorionic membrane. Immediately following this the spindle for the second division develops, and the division proceeds to the metaphase. In this stage it remains until after fertilization.

The Tertiary Egg Coverings. — As the egg passes down the oviduct from the infundibulum to the uterus the walls of the duct secrete about it three or four layers of albuminous material which constitute a tertiary covering. These layers are hardly distinct as such at this time, but as will appear below they become so after contact with the water.

Spawning. — Within about two hours after entering the infundibulum the egg reaches the uterus where it may remain for a day or two until this portion of the duct is full. The accumulated mass of ova are then expelled into the water, and in the common American Wood Frog a single such act of expulsion usually completes the process of spawning. In some varieties of Frog, however, the expulsive act is followed by another accumulation of eggs, and the spawning period is thus prolonged. Hence, though in American Frogs its duration is usually not more than a few days, in some European species it may continue for over a week, the process in any case being retarded by cold. As already noted the male remains in amplexus throughout this time, although in those instances where repeated expulsions are the rule, the actual extru-

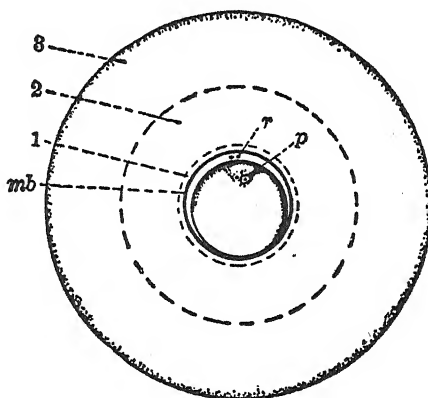


Fig. 59. — Egg of Frog a short time after laying and fertilization, showing the swollen egg membranes. From Ziegler (*Lehrbuch*, etc.), after O. Schultze.

mb. The chorion, presumably plus the vitelline membrane. *p.* Pigmented penetration path of the spermatozoon. *r.* Polar bodies lying in perivitelline space. 1, 2, 3, inner, middle and outer albuminous membranes or layers of "jelly."

sion of eggs generally occurs only in the early mornings of successive days. In this way he is always in a position to discharge sperm over the ova as they emerge. Furthermore, although this act of amplexus has been shown to have nothing whatever to do with ovulation, it is now clear, as intimated above, that it does afford the stimulation for spawning. Without it "stripping" of the female is necessary in order to press the accumulated eggs out of her uteri. The total number of eggs spawned in a season varies in different species of Frogs and in different individuals. Thus in *Rana temporaria* it runs from 1000 to 2000, while in *Rana esculenta* it may be anywhere from 5000 to 10,000.

It is of some interest in this connection to note the factor which is the stimulus for amplexus on the part of the male. It might be assumed to be the presence of the female, or at least of a female with eggs in her uteri. Such, however, is not the case. As again clearly shown by Rugh ('37) this action on the part of the male Frog is, like ovulation in the female, entirely conditioned by a secretion of the anterior pituitary. Indeed not only does the secretion of his pituitary cause him to go into amplexus with a female Frog, or any other convenient object, but it also brings about the release of ripe sperm from the Sertoli cells of his testis. Without an adequate increase in this hormone on the other hand, the male shows no interest in a female even though her uteri may be filled with eggs.

The Effect of Water on the Tertiary Membrane.—After spawning the membrane indicated above of course comes in contact with the water, and by absorbing it, begins immediately to swell. This action progresses rather rapidly at first, so that within two or three minutes the jelly-like covering has increased from one sixth the diameter of the egg to about one half that diameter. In fifteen minutes it generally equals the egg diameter: thereafter the swelling becomes slower. At this point, if fertilization has not occurred the absorption of water by the jelly is said almost to cease. If fertilization has taken place, however, the swelling process may continue for several hours until the thickness of the jelly is as much as twice the width of the ovum.

This thickening reveals more clearly the three or four layers of which the jelly membrane is really composed. The innermost is a thin dense stratum applied closely to the chorion, and sometimes erroneously referred to as the chorion itself. Next comes a rather thick and watery layer, and finally one which is both thick and firm. When a fourth is present it is thin and fibrous; it does not occur outside, but just beneath the thick firm layer which is always outermost.

Although some species of Frogs have elaborate habits connected with the care of the eggs, the common Frog does not. When fertilized, the eggs are simply deposited and left to their fate. On this account the thick envelope of jelly which they possess appears to exercise several important functions. In the first place it serves to attach them to each other and to debris, so that they are not readily washed about. It protects them from mechanical injury, and also appears to be distasteful to water snails and perhaps other animals.

In addition to these functions it has long been claimed that the jelly serves as a lens to concentrate the rays of the sun upon the eggs, and thus to raise their temperature. This it was assumed would be of advantage because it would speed up the otherwise slow development in the cold water of early spring. This particular claim and assumption, however, is an excellent example of the way in which an untested assertion which seems superficially reasonable, may become widely accepted, and yet be entirely without foundation in fact. Thus to begin with, *Rugh* ('33) showed that temperatures a little too high will injure the eggs, and we know from other sources (see below) that such temperatures upset the sex ratio. Hence it would appear probable that the risk accompanying such an effect as suggested would more than overbalance any possible advantage. Be that as it may, *Rugh* has further shown that the water in which the eggs occur, plus the jelly, which is about 78 per cent water, filters out most of the radiant energy of a heat-producing character. Consequently the light which the eggs receive, even though it is absorbed by the black pigment on their surface, produces relatively little heat. Lastly *Cornman* and *Grier* ('41) have demonstrated very conclusively that even if there were any heat in the light passing through the jelly, the latter totally lacks the effect of a lens. Indeed its refractive index is about that of the water in which it occurs, and hence with the curvatures involved would bring the light to a focus far beyond the egg. Thus it would appear that far from raising the temperature of Frog eggs the jelly may even act as an insulator to keep them from getting too warm.

FERTILIZATION AND EGG SYMMETRY

FERTILIZATION

The Penetration of the Sperm. — As the eggs are extruded by the female, the male Frog immediately discharges over them the seminal fluid. This fluid contains thousands of spermatozoa, and hence the eggs

tend to be surrounded by them. Many of these pierce the outer jelly, but usually one of them is slightly in advance of its fellows and thus arrives first at the surface of the egg itself. As soon as it has started to enter some change is effected in the egg so that the remaining sperm are unable to pass beyond the jelly. Polyspermy is thus abnormal in the Frog and when it occurs the course of development is interfered with.

The entrance of the sperm always occurs in the animal hemisphere of the egg, and usually, according to some authorities, about 40° from the pole. Aside from these limitations, however, there is apparently nothing which fixes the point of penetration; that is, this point may be located on any one of the infinite number of meridians which may be imagined to pass from one pole of the egg to the other.

The Perivitelline Space. — The penetration of the ovum by the sperm seems to cause the egg to give up a certain amount of its fluid. In any case, whatever its source, fluid does collect at this time between the chorion and the surface of the ovum. It is indeed presumably inside the vitelline membrane if the latter exists, and hence the space containing this fluid is as usual termed the *perivitelline* space. Its formation releases the egg from the grip of its coverings so that it is free to rotate within them. Under these conditions if the lighter animal pole is not already uppermost it presently becomes so.

The Entrance Path. — In the case of the Frog the whole spermatozoan enters the ovum, and it usually requires a minute or two for it to get entirely inside. The tail then disintegrates, and the head and middle piece travel steadily along a path which is generally approximately a radius of the egg, leaving a trail of pigment behind them (Fig. 60, A). This is the *penetration* or *entrance path*, and as the head and middle piece move along it, the usual rotation of these parts occurs, thus placing the latter structure in the lead. At the same time the head is enlarging to form a typical nucleus.

The Second Maturation Division. — Meanwhile the stimulus of the entrance of the sperm has incited the completion of the second maturation division of the egg nucleus which had paused in the metaphase. After throwing off the second polar body, the egg nucleus withdraws from the surface of the ovum, usually to a position in the egg axis. The sperm nucleus then proceeds toward it.

The Copulation Path and the Fusion of the Egg and Sperm Nuclei. — As suggested under the general topic of fertilization, the course followed by the sperm immediately after its penetration of the egg (i.e., the entrance path) may not be directed exactly toward the egg

nucleus. In those instances where it is not, therefore, the point where the sperm does start to move directly toward this nucleus is marked by a slight change in its course. The second portion of the sperm path which thus arises, as has already been noted, is then called the *copulation path*, and like the first portion, in the case of the Frog, it is marked by a trail of pigment (Fig. 60, *A*).

Proceeding along this second path the sperm nucleus presently meets that of the ovum. Meanwhile the middle piece has initiated the formation of a division-center and aster, and before the meeting of the pronuclei occurs this new center and its aster have divided into two. The division has taken place at right angles to the copulation path, and hence as the nuclei come together the axis joining the division-centers coincides with their plane of union (Fig. 60, *A*, *B*).

THE SYMMETRY OF THE OVUM AND ITS SIGNIFICANCE

The causes which determine the symmetry of any ovum and the relation which this symmetry bears to cleavage and to the symmetry of the embryo are subjects of fundamental importance for the understanding of development. They have therefore received considerable attention in different groups of animals, and among Vertebrates the Frog's egg has seemed particularly well adapted for such study. Hence it appears desirable in the case of this animal to make some mention of the results to which this study has led. It must be noted, however, that in spite of the work which has been done, there still exists some disagreement as to the exact facts, at least as regards certain details. In the interest of clearness, therefore, it seems best merely to state the main features of this phase of development in the Frog according to one view, the accounts followed being chiefly those of Roux and Jenkinson.

The First Plane of Symmetry.— Before the egg is fertilized it is radially symmetrical about an axis passing through its poles. The penetration of its surface by the sperm, however, confers upon it a bilateral symmetry. That is to say, the point of this penetration, together with the polar axis, determines a plane which, save for the possible eccentricity of the egg nucleus, divides the ovum into equal halves. It may be termed, therefore, the *sperm entrance point plane* (Fig. 60, *A*). The existence of this plane of symmetry, determined solely by the egg axis and the sperm entrance point, however, is brief. Other factors presently enter which determine a second plane, often, though not necessarily, closely correlated with the first (see below), and developed in the following manner:

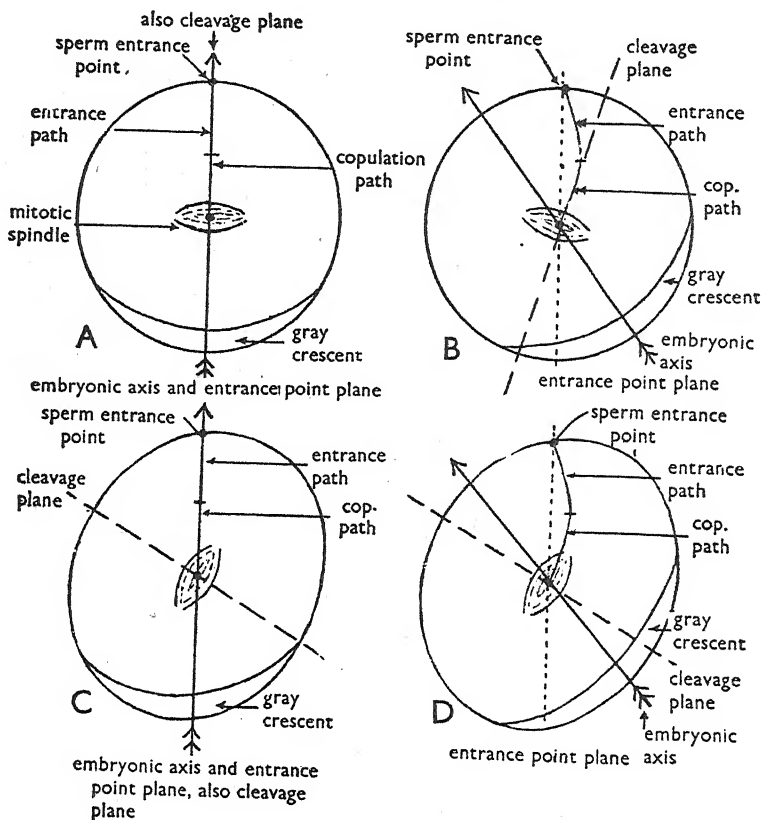


Fig. 60.—Diagram to illustrate some of the possible relations of the axes in a fertilized Frog egg. In all cases the egg is assumed to be viewed from the animal pole. The arrow indicates the longitudinal axis of the future embryo, with the head pointing anteriorly. The dash line indicates the first cleavage plane where the latter does not coincide with the longitudinal axis of the future embryo. The dotted line indicates the entrance point plane where this does not coincide with the longitudinal axis of the future embryo. The dot in the center indicates the center of the animal pole.

A. An egg in which the entrance point plane, the entrance and copulation paths, the gray crescent plane, the first cleavage plane and the longitudinal axes of the future embryo all coincide. B. An egg in which the entrance path and the copulation path are not in the same straight line. Hence the gray crescent plane and the longitudinal axis of the future embryo fail to coincide with either the entrance point plane or the first cleavage plane. C. An egg distorted by pressure. Note the consequent orientation of the mitotic spindle as explained in text. This prevents coincidence of the first cleavage plane with any of the others. D. The same situation with the added complication due to the fact that as in B the entrance path and copulation path are not in the same straight line. Note that in all instances the gray crescent plane and that of the longitudinal axis of the future embryo coincide.

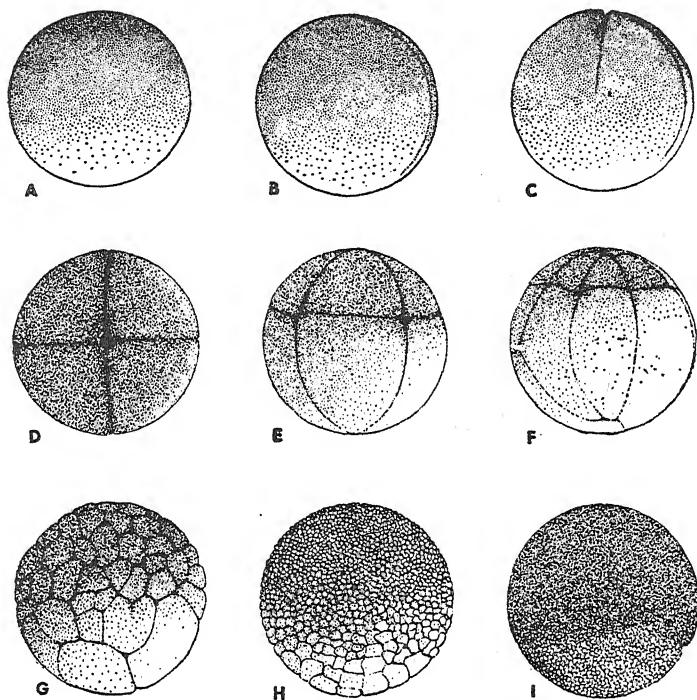


Fig. 61.— Cleavage stages and the beginning of gastrulation in the Frog's egg (*Rana pipiens*). The shading in this figure indicates the distribution of pigment, except along the lines of cleavage, where as usual it denotes shadow. *A*. Fertilized egg viewed from the left side in terms of the future embryo. Note the left half of the gray crescent at the right side of the figure, i.e., its dorsal side in terms of the embryo. *B*. An egg in which the first cleavage has been almost completed. Since the egg is again being viewed nearly from its left side in terms of the future embryo, the cleavage furrow is virtually in the plane of the paper and scarcely shows. The gray crescent is again to the right as in *A*, but at this stage the region of the crescent has evidently become whitened and so added to the original light area of the vegetal pole. *C*. An incomplete four-cell stage, also viewed from the left side. The second furrow has not quite reached the vegetal pole. *D*. A view of *C* from the animal pole, with the region of the gray crescent toward the right (dorsal). *E*. An eight-cell stage. The animal pole is again at the top of the page, and the vegetal pole at the bottom, but the future dorsal region is turned slightly toward the observer, thus showing part of the first furrow. *F*. An approximate sixteen-cell stage directly from the left side. The cleavage is obviously somewhat irregular. *G*. Between a 64- and 128-cell stage viewed from the left side. *H*. A virtually complete blastula from the left side. Note that the pigmented area is tending to move downward somewhat. *I*. An early gastrula from the left side. The cells in the animal hemisphere are too small and numerous to indicate separately. The beginning of the blastopore lip is visible as a slight notch in the lower right side of the figure.

The Second Plane of Symmetry.—As the sperm travels along the first part of its path within the egg, it seems to cause certain disturbances in the egg substance. The result is a more thorough separation between yolk and cytoplasm, and an apparent streaming of the latter in the direction of the sperm. This flow seems to cause a withdrawal of pigment granules from along the border of the pigmented animal hemisphere on the side of the egg from which the flow is taking place, i.e., the side approximately opposite to that upon which the sperm entered. The result is the appearance upon that portion of the pigmented border of a lighter strip termed the *gray crescent*. This crescent is usually quite clear shortly after fertilization and during the first few cleavages. After a little time, however, its outlines become less distinct. Hence its existence is soon detectable only by the fact that the light area extends somewhat higher up on the side of the egg where a definite crescent originally occurred (Fig. 61). The new plane of symmetry, therefore, is one which again passes through the egg axis and also bisects the gray crescent, or the increased area of white which replaces it. It may be called the *second* or *gray crescent plane*, and by virtue of its method of formation it will evidently have a decided tendency, as suggested above, to coincide with that of the sperm entrance point (Fig. 60, A). That this is a tendency rather than an inevitable condition, however is due to the following considerations:

It will be recalled that the path of a sperm toward the egg nucleus is not necessarily a straight one. Presumably because of failure to enter at quite the correct angle, the sperm may not at first be headed in the right direction, and hence has to alter its course, thus producing the initial or entrance path and the later copulation path. But, as suggested above, it turns out that the influence of the sperm in causing the pigment withdrawal is largely exerted as it passes along the entrance path. Therefore, if the entrance path does not happen to lie in a vertical plane coinciding with the poles and a radius of the egg, it follows that in such cases the entrance point plane and the gray crescent plane also will not coincide (Fig. 60, B).

The Cleavage Plane.—Following the union of the egg and sperm a third plane makes its appearance, i.e., that of the first cleavage. This incidentally is of course an actual plane, not merely a hypothetical one determined by three points. Under normal conditions this plane passes approximately through the animal and vegetal poles, a condition resulting from the following facts:

In accordance with a generalization known as Hertwig's law the

mitotic spindle always tends to lie so that its longitudinal axis coincides with that of the yolk-free cytoplasm of the cell. Now in the Frog egg this yolk-free cytoplasm ordinarily occupies about the upper third of the animal hemisphere, and hence has approximately the form of a rather thick plano-convex lens. Therefore the long axis of the spindle may fulfill Hertwig's law by lying in any direction so long as it is parallel to the flat surface of the lens-shaped disc of cytoplasm. This will of course also make it at right angles to the polar axis of the egg. Furthermore, since the egg nucleus is in this axis the movement of the sperm and spindle to that nucleus will presently cause the middle of the spindle to coincide with the egg's polar axis. Finally because the plane of cleavage is perpendicular to the length of the spindle at its middle, this plane will also coincide with the egg's polar axis and so pass through its poles (Fig. 60, *A, B*).

Though Hertwig's law thus determines that the first cleavage must pass through the egg poles, this law does not determine with which of the infinite number of imaginary radii emanating from the polar axis the cleavage must coincide. There is another consideration, however, which does determine the radial direction of this cleavage. The sperm division center, it will be recalled, divides so as to cause a new mitotic spindle to form at right angles to the copulation path. Hence the cleavage plane should coincide with this path, as well as pass through the poles of the egg. Under most circumstances these are the only factors involved, and such coincidence occurs (Fig. 60, *A, B*). It should be noted, however, that pressure on the egg perpendicular to its polar axis may distort the lens-shaped disc of cytoplasm so that its periphery is no longer circular. Under such conditions the mitotic spindle, in accordance with Hertwig's law, will be displaced so that the cleavage plane may not be related to any other (Fig. 60, *C, D*).

The Plane of Embryonic Symmetry.— This plane is of course the one which divides the future embryo into equal right and left halves. In the Frog it always coincides with the gray crescent plane (Fig. 60), i.e., except when the latter fails to exist (see below). This coincidence results from the fact that normally the dorsal blastoporal lip develops at the middle of the lower border of the crescent. On this basis one might assume that the median plane is determined by the gray crescent, the latter having been in turn determined by the entrance path of the sperm. Indeed this has been quite generally regarded as true. As parenthetically suggested above, however, it must now be stated that the existence of a gray crescent is not inevitable. Thus the writer has ob-

served fertilized eggs in which the pigment merely tapered off in streamers more or less equally distributed on every side. Yet many of these eggs appeared to develop quite normally. It should be added that these were eggs which had been obtained by stripping pituitary injected females, and which had then been artificially inseminated. Whether this lack of a gray crescent ever occurs in eggs normally produced the author cannot say, but it seems not unlikely that it does. Indeed this seems highly probable in view of the fact that in some Amphibian eggs there is no pigment from which a crescent can be formed, and yet needless to say, these eggs develop an embryonic symmetry.

In view of these facts, then, the question arises as to what if any relation the gray crescent, when it exists, really does have to embryonic symmetry, since, under some circumstances, the latter can quite evidently develop without it. The most probable explanation of the situation seems to be this: The passage of the sperm along the entrance path causes a rearrangement of materials within the egg with a certain reference to this path. Of this there seems little doubt. Normally, moreover, this rearrangement involves the withdrawal of superficial pigment in the eggs of those Amphibians which possess it, and thus produces the gray crescent. However, the two phenomena, i.e., withdrawal of pigment and rearrangement of internal materials, are not inevitably connected, and it is the latter which is fundamentally significant: Hence it would appear that the entrance path of the sperm is the initially determining factor of embryonic symmetry in fertilized Amphibian eggs. What this factor may be in eggs artificially stimulated to parthenogenesis is at present unknown. Also, what may happen to the initially determined symmetry in eggs later abnormally oriented remains to be stated (see below).

Relationship of the Various Planes Summarized. — There have now been defined four planes, the sperm entrance point plane, the gray crescent plane, the first cleavage plane, and the plane of embryonic symmetry. Of these four the one most frequently out of line with the others is that of the sperm entrance point. This is because, as shown, the other planes are all related in one way or another to the path, or paths of the sperm, and not essentially to its point of entrance. Thus the gray crescent plane is determined by the entrance path. The cleavage plane in turn is fixed by the copulation path in conjunction with the shape of the yolk-free cytoplasm and its relation to the egg poles. The plane of embryonic symmetry normally coincides with that of the gray crescent, but this is probably not a causal relationship. The really fundamental determiner of embryonic symmetry under normal conditions is

probably the path of sperm entrance. In conclusion it may be stated that there will be a considerable tendency for all four planes to coincide (Fig. 60, *A*).

CONCLUSIONS DERIVED FROM EXPERIMENTS

It is of interest in connection with the question of the relation of embryonic symmetry to the cleavage and gray crescent planes to note the results of certain experiments which have been performed upon the two cell stage of the Frog and other Amphibians. It is not possible to kill or remove one blastomere of the egg of the common Frog without killing the other. It has been found, however, that if a hot needle is thrust into one of the cells, this cell though not dead will fail to divide. Under these circumstances it was long ago discovered by Roux ('88), Morgan ('02, '04), Hertwig ('93), and others, that when this is done to eggs in which the first cleavage plane has passed through the middle of the gray crescent, the uninjured cell may continue to develop. Under these conditions it then generally produces approximately the lateral half of an embryo, with the undeveloped hemisphere of material comprising the other blastomere adhering to it (Fig. 62). Another investigator, McClendon

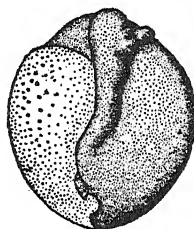


Fig. 62.—A half embryo of the Frog produced by thrusting a hot needle into one of the first two blastomeres. After Roux.

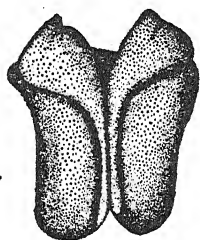


Fig. 63. — Two Frog embryos united back to back, produced by inverting the two-cell stage. After Schultze.

('10), then found that in the case of the tree frog, *Chorophilus*, it is possible by the proper technique to remove one of the first two blastomeres without injuring the other. When this was done it was discovered that the remaining cell developed not into a half embryo as in the preceding experiment, but into a whole one. Taken together these results might reasonably be interpreted to mean that the failure to develop a whole embryo in the first case was due simply to the inhibiting presence of the inert blastomere, and indeed McClendon himself did reach this conclusion. Other facts exist, however, which render another interpretation more probable. They are as follows:

It was discovered by Schultze ('94) that if the egg of the Frog is exactly inverted following the first cleavage, and held in this position, each blastomere will give rise to approximately a whole embryo, the

two animals being united, however, in various degrees after the manner of Siamese twins (Fig. 63). This interesting result was supplemented by an experiment by Morgan ('95) in which he inverted the two cell stage after the manner of Schultze, but in addition injured one of the blastomeres. Under these conditions the remaining blastomere instead of developing into a half embryo as in the first experiment, formed a virtually whole one, despite the presence of the injured hemisphere. The latter, therefore, cannot be the cause of the half embryos. More detailed observation of what takes place in the inverted cells, however, seems to furnish a possible explanation of the results in all the above cases.

It has been noted by several observers that when the eggs are inverted the contents of the cell or cells becomes rearranged in response to gravity. Thus the materials of the gray crescent can sometimes be seen to become separated into two parts. At the same time the lighter yolk free cytoplasm comes to what is now the top (the former vegetal pole), and the heavier yolk sinks to the former animal pole. With such profound changes going on there is every reason to believe that the critical materials concerned with embryonic symmetry are also rearranged, and probably divided. If this is so it might be expected that with their division two embryos would develop, as in fact they do. As regards McClendon's isolated, but uninverted blastomeres, it must of course be supposed, according to this hypothesis that a similar reorientation takes place, though in these cases it must presumably occur either as a result of the manipulation of the eggs, or on account of the change in shape of the isolated cells.

CLEAVAGE, GASTRULATION, AND THE FORMATION OF MESODERM, NOTOCHORD, AND MEDULLARY PLATE

It has already been suggested that in the Frog the character of the processes indicated is transitional; it serves to bridge the gap between the activities observed in the development of *Amphioxus* and those in some of the forms which are to follow. Not only is this true, but the character of the Frog's egg as regards its yolk content is also transitional. The egg of *Amphioxus* was telolecithal, but the amount of yolk was relatively slight. The egg of the Frog is telolecithal, but the amount of yolk is much greater. Finally, as will be seen, this condition is carried to its extreme in the Fish and Bird. As our study of these forms proceeds it will become increasingly apparent that this parallelism between the character of early development and the yolk content is

not a coincidence. Rather, as intimated in the first chapter, the latter very largely determines the former. The student then should keep this clearly in mind in attempting to understand the stages which follow as compared with corresponding stages in *Amphioxus*.

CLEAVAGE

The Early Stages. — In spite of the larger amount of yolk in the Frog's egg, segmentation is still holoblastic. Following the second cleavage, however, it is less nearly equal than in *Amphioxus* (Fig. 61). As has been stated the first division plane normally passes through the poles of the egg, and is thus perpendicular to the egg equator, and vertical if the egg is normally oriented. This means that it divides the ovum into parts which are at least quantitatively similar. The particular meridian cut by the division is determined by factors noted above. The furrow which marks the beginning of this cleavage appears on the upper surface of the ovum about two and one half hours after fertilization and within an hour has extended around to the ventral pole. By the time it has reached this pole, the internal substance of the egg is also divided.

A period of "rest" ensues, and then, about three quarters of an hour after the appearance of the first division, the furrows of the second become evident. This cleavage is also vertical and at right angles to the first. The furrow in each of the two hemispheres again begins approximately at the animal pole, often exactly so. When the latter is the case the upper ends of these furrows will evidently lie opposite each other and form a continuous line across the pole (Fig. 61, *D*).

Following the completion of the second cleavage, the third soon starts. It is horizontal, and in each of the four cells it lies about 60° below the animal pole. Hence its furrows form a virtually continuous line around the egg a little above the equator. This is the typical or at least the ideal condition (Fig. 61, *E*). There are, however, not infrequent variations.

The furrows of the fourth cleavage are in general vertical, and tend ideally to meet one another at the poles. This tendency, however, is seldom perfectly realized, even in the animal hemisphere. Thus in the latter half, the lines of division usually pass either to one side or the other of the polar center, while in the vegetal hemisphere this and other irregularities are even more marked. The ideal result, however, is sixteen cells, eight relatively small pigmented ones above, and eight larger whitish ones below (Fig. 61, *F*).

The fifth cleavage, resulting in the formation of thirty-two cells, is

still more variable than the fourth. There is a tendency, however, for the furrows to be horizontal, and to form four tiers of eight cells each. In the most regular instances the cells of the two upper tiers are about equal, and are all pigmented. The cells of the third tier are about mid-

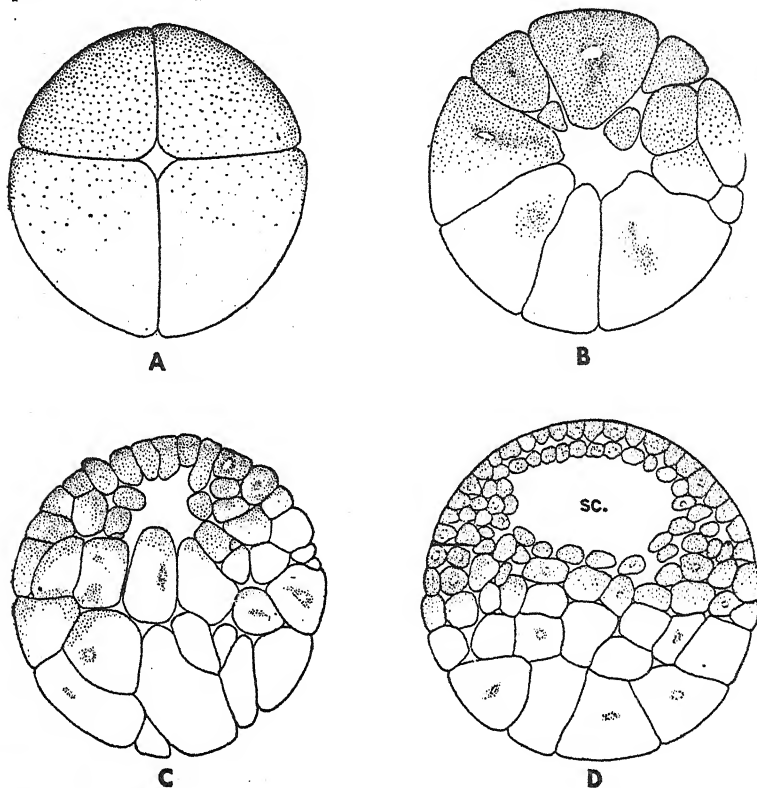


Fig. 64. — Median vertical sections of four cleavage stages in the Frog's egg. *A.* An eight-cell stage. Note the small segmentation cavity or blastocoel. *B.* A later stage (about 32 cells) which may be called an early blastula. *C.* A later blastula. *D.* A still later blastula, showing marked increase in size of segmentation cavity.

way in size between those above and those below them. They are approximately on the equator, and contain less pigment than the two upper tiers. The lowest tier is formed of the largest cells, which are mostly without pigment.

The Blastula. — By the time the thirty-two-cell stage is reached it is hardly possible longer to refer to this dividing sphere as an egg. It may now, therefore, be termed the *blastula*. Within this *blastula* is the *blastocoel* or *segmentation cavity*, which arises as follows:

From the first the cells into which the ovum has been divided are pressed rather closely against one another so that their surfaces of contact are flattened. This, it will be recalled, is contrary to the rounded condition of the very early cleavage cells of *Amphioxus*. Even in the Frog, however, the inner ends of the cells show some curvature, and by about the eight to the sixteen cell stage these inner ends are sufficiently rounded so that they are no longer in contact. Thus is produced the blastocoel, which, because of the smaller size of the cells at the animal pole, is somewhat above the equator of the blastula (Fig. 64). Also up to the beginning of gastrulation the blastocoel is gradually increasing in size, due partly perhaps to the closer packing of the cells, to the secretion of albuminous fluid from them, and to the infiltration of water from without (Fig. 64, A, B). The latter two factors are probably the more important.

Besides this increase in size of the blastocoel, cleavage following the thirty-two cell stage becomes quite irregular, and cells begin to be split off internally. At the same time the relatively yolk-free cells of the animal hemisphere begin to divide much faster than those of the vegetal hemisphere, and some of the smaller ones tend to migrate toward the equator, thus making the roof of the blastocoel thinner. Regarding the matter of the cleavage rate in general, an interesting fact has been noted by Ting, '51. He found by crossing different species of Frogs, using both normal and enucleated eggs, that the rate of division up to the time of gastrulation is determined entirely by the egg cytoplasm, whose character was presumably previously determined by maternal genes.

Finally, at what may be termed the end of the blastula period, the following conditions obtain: First the blastula is about one fifth larger than the original egg, the increase in size being mainly due no doubt to the absorption of water noted above. Secondly, the superficial pigment has everywhere extended downward somewhat, thus decreasing the white area (Fig. 61, H). This extension having been approximately uniform, however, the latter region still reaches farther upward upon the side where it was originally augmented by the addition of the gray crescent. Thirdly, sections reveal the fact that on the side opposite to that which was marked by the gray crescent, the wall of the blastocoel is usually slightly thicker than it is elsewhere (Fig. 67, A). Lastly, it may be noted that a split has occurred in the roof of the segmentation cavity, so that this wall is composed of two sheets. The outer is the *epidermal layer*; the inner is called the *nervous layer* because parts of it help form the nervous system.

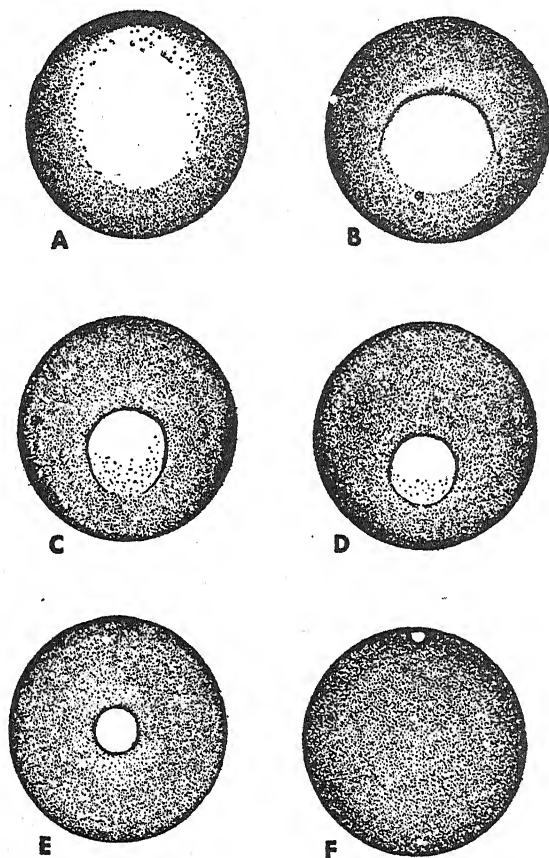


Fig. 65. — Diagrams of the closure of the blastopore in the egg of the common Frog (*R. temporaria*). From Jenkinson (*Vertebrate Embryology*). In A-E the egg is viewed from the vegetal pole, and in F, speaking in terms of the future embryo, from its ventral side. The dorsal lip is at the top of the figures. In D the ventral lip has just been formed and the blastopore is circular. In E the rotation of the whole egg has begun, and in F is complete.

GASTRULATION

External Processes. — Upon the side of the blastula where the white area was increased by the addition of the region of the gray crescent, it has been noted that the pigment is still not quite so far down as upon the side opposite. Nevertheless, even at the former point the pigment extends markedly below the equator, the line between the light

and dark zones being everywhere marked by an area of intermediate shading. It is then midway between the ends of the former crescent region, and toward the lighter and lower side of the shaded area in this region that the dorsal blastoporal lip first appears. It is thus probably located at approximately the lower border of the original crescent, though the exact relation is difficult to determine because of changes in pigmentation during cleavage. It is also somewhat below the level of the floor of the blastocoel. This lip has the appearance at first of a small dent, which soon elongates into a groove following roughly the border of the pigmented area (Fig. 61, *I*; Fig. 65, *A, B*; Figs. 67, *B* and 68, *B*).

As the process of elongation continues it is accompanied externally by two phenomena. In the first place the groove gradually extends around either side of the gastrula, and as it does so the pigment advances to its edge, i.e., to the lip of the blastopore. This lip thus comes to constitute a sharp boundary between the dark and light areas (Fig. 65, *A, B, C*). In the second place the blastoporal lip everywhere moves steadily nearer to the vegetal pole. This movement is greatest on the side where the groove first appeared, i.e., at the dorsal lip, and becomes progressively less toward either side. The first process, i.e., that of lateral extension, causes the groove to become curved so that it has the shape of a crescent, and eventually the horns of this crescent meet each other so as to form a complete circle. A continuation of the second process, i.e., the downgrowth of the lip, and hence also of the pigmented area, then results in a rapid diminution of the white region. Thus the latter is soon in the form of a circular spot which is being encroached upon from all directions (Fig. 65, *D, E, F*).

Epiboly. — The white region evidently occupies the position of the *blastopore*. The first appearance of the groove marks the beginning of overgrowth by the dorsal blastoporal lip, while the lateral extensions of this groove indicate the same process on the part of the lateral lips. Finally, as already noted, the ends of the grooves meet one another on the future postero-ventral side of the *gastrula*, and thus show that there also a slight downgrowth is taking place. This overgrowth of the yolk, or epiboly, by the cells of the blastocoel roof necessarily involves the use of material which can only be supplied by a thinning of this roof due to a rearrangement of its cells (Fig. 67).

Convergence. — In correlation with epiboly certain other processes are also occurring, for an understanding of some of which more than mere external observations are required. There is one other, however,

which can also be studied from the outside. Such a study has proven especially fruitful in the case of some of the tailed Amphibians, like Triton, in which the egg is relatively colorless. In these animals it is thus possible to put stains upon the outside of the blastula, and so to observe what movements occur there during the ensuing gastrulation. This was done by Vogt ('22, '25, '26) and Goerttler ('25) who placed pieces of agar saturated with stains upon the egg membranes. The stains penetrated the membranes and colored the cream tinted surface of the late blastula. The results are depicted in Figure 70, *A, B, C, D*. From these it appears that there is a streaming of the materials of the dorsal and lateral surfaces of the early gastrula toward the blastopore. At the same time, as is especially indicated by the later stages (*C* and *D* of Fig. 70), there is a shifting of the lateral regions toward the mid-line. It is this combined type of movement which is now generally described as *convergence*, and though it has some aspects of the old alleged concrescence it is obviously not the same thing. Thus it is evident that in this case, as in *Amphioxus*, the lips of the blastopore do not actually constitute the sides of the embryo, or even furnish much of the material for it. However, a good deal of this material does as usual pass over the lips, and for this, and perhaps merely historical reasons, they are sometimes referred to in this animal as the *germ ring*.

More recently Schectman ('42), Holtfreter ('43) and others have made further studies of the movements thus described in an effort to arrive at a more basic understanding of them. Schectman particularly stresses the idea that none of the regions undergoing the movements heretofore indicated act entirely independently. Each has certain autonomous capacities, such as the extension or self-stretching capacity of the presumptive chordal region of the dorsal blastoporal lip. This region, however, lacks "invagination" (involution) capacity which is conferred on it by the normally adjacent lateral lips. The combined movements resulting in these regions Schectman therefore calls "correlative." Holtfreter has sought especially to reach physico-chemical explanations of the gastrulation phenomena. Thus he has suggested that an unfolding of denatured protein molecules is partly responsible. This unfolding, it is thought, causes a spreading of the superficial cells over a substrate with appropriate adsorption properties. The epiboly and perhaps the convergence are hence due to this spreading tendency, which is apparently augmented by a lowering of surface tension in parts of the spreading cells. It will be recalled that such a change in surface tension was also referred to in the general discussion of gastru-

lation as a possible cause of involution and invagination. On the basis of these conclusions it is further suggested that all these cell movements may be essentially similar to the cell movements seen in wound healing and in phagocytosis. Additional study is of course needed either to disprove or to confirm and amplify these ideas.

Rotation.—Returning to more obvious and directly observable matters, we are confronted with a very definite change in the position of the whole gastrula which accompanies the processes just described. The

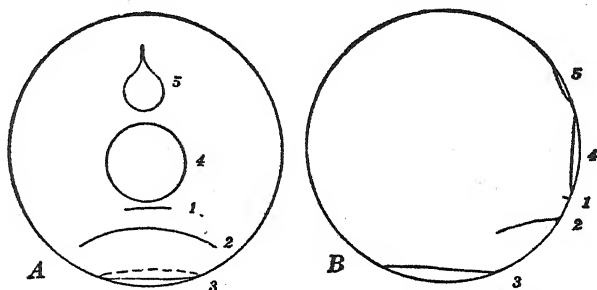


Fig. 66.—Diagrams of the Frog's gastrula showing the position of the blastopore at various ages. From Kellicott (*Chordate Development*). A. Posterior view. B. Lateral view. 1-5 indicate the successive positions and forms of the blastopore. The change in position is due both to the actual growth movements of the blastopore, and to the rotation of the entire gastrula.

movement of epiboly continues until the dorsal lip has passed over an arc somewhat greater than 90° , and the area of white, i.e., the blastopore, is reduced to a small circle. This area, therefore, will be situated rather beyond the original vegetal pole. It is now to be noted, however, that accompanying this downgrowth of the dorsal lip another and quite different movement has also been going on. The entire gastrula has been rotating about a horizontal axis lying at right angles to the original median plane of the egg. That is the direction of rotation is such that the dorsal lip is in a sense carried backward in one direction as fast or faster than epiboly moves it forward in the other. The result is that at the completion of both processes the blastopore, formed at approximately the vegetal pole, is posterior, and the morphologically dorsal and ventral lips are actually dorsal and ventral (Fig. 66). From this it also follows that the original animal pole of the egg is to form the antero-ventral side of the future embryo, while the region formerly marked by the gray crescent is to form the dorsal side.

As regards the events so far described it is evident that gastrulation

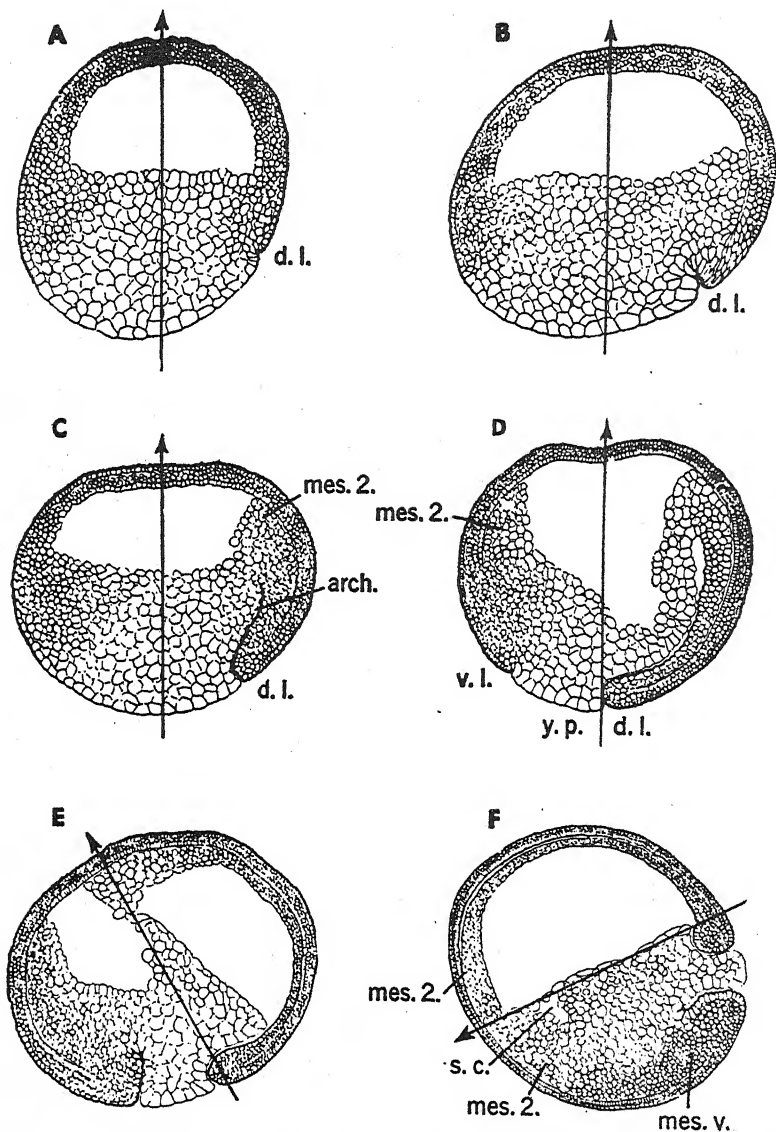


Fig. 67. — Sagittal sections through Frog's egg during formation and closure of blastopore. From Jenkinson (*Vertebrate Embryology*). A-D. Before rotation. E During rotation. F. After rotation. The arrow marks the egg-axis, its head the animal pole. arch. Archenteron. d.l. Dorsal lip. mes.v. Mesoderm originating at ventral lip (i.e., a very small part of that which is classed as peristomial). mes.2. Mesoderm originating from the yolk cells pushed into segmentation cavity (i.e., gastral). s.c. Segmentation cavity. v.l. Ventral lip. y.p. Yolk plug.

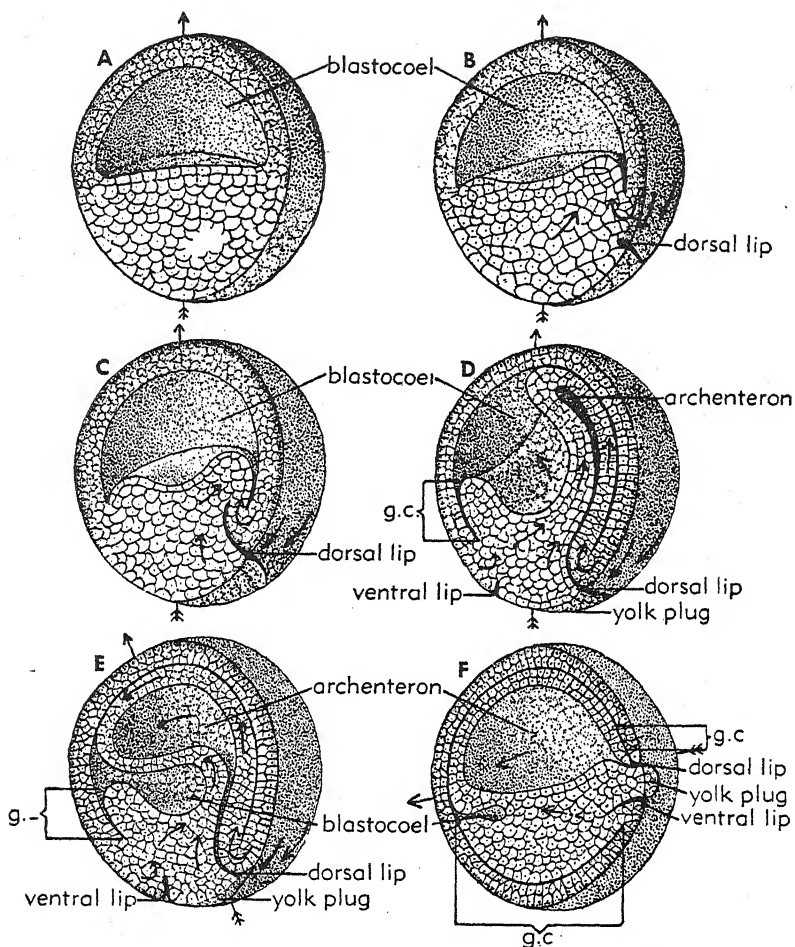


Fig. 68. — Semi-diagrammatic stereograms of a hemisected Frog blastula, A, and successive stages of hemisected gastrulae, with small curving arrows indicating the directions of cell movements. Stages in alphabetical order.

Arrows on outer uncut surface of the gastrulae, to the right in each figure, show movement of material toward blastoporal lip. *Involution* of material over lip shown by arrow on cut surface of lip margin. *Invagination*, of a sort, shown by arrows on cut surface of yolk mass and floor of archenteron. *Epiboly*, evident from decrease in size of yolk plug. *Delamination*, in this case gastrular cleavage, shown by extent of splits bracketed under letters g.c. Ingression, being most questionable, not shown, but would be designated by arrows pointing directly from vegetal pole to floor of blastocoel in early stages. Animal pole marked by head of arrow outside each figure. *Rotation* of entire gastrula shown by changes in the positions of the poles in E and F.

in the Frog is not essentially dissimilar to the same process in *Amphioxus*. The main differences are due to the presence of the large yolk cells. Thus, to cite one instance, if these were absent the blastoporal lip would bound an opening just as in the former case. Here, however, this opening, i.e., the blastopore, is filled by these cells, which at this point are therefore termed the *yolk-plug*. As will presently appear, the phenomenon of rotation and various internal peculiarities are also due to the presence of so much inert nutrient material.

Internal Processes. — While the above changes are apparent from the outside of the gastrula, sections through it at various stages will reveal important accompanying developments within. They are as follows:

Invagination. — As the external processes of gastrulation begin, meridional sections of the blastula (or early gastrula) bisecting the future dorsal blastoporal lip reveal the fact that the floor of the blastocoel is beginning to move upward. Usually this movement begins on the dorsal side nearest the dorsal lip, and spreads part way around the margins of the blastocoel in company with the external extension of the lateral lips (Figs. 67, *B*; 68, *B*). Sometimes, however, the up-pushing is more central, and thus causes the blastocoel to become crescent shaped. In either case the movement is essentially one of invagination, albeit an invagination which is considerably hindered and modified by the mass of material to be moved. This mass of course is the yolk which occupied the vegetal half of the egg, and now occupies the relatively large and numerous vegetal cells. This modified invagination continues until the blastocoel cavity has been virtually eliminated, except for the narrow slit separating the outer layer of cells, now *epiblast* from the inner yolk-filled cells, now *hypoblast* (Fig. 67; Fig. 68, *C, D, E, F*).²

² It seems pertinent to mention at this point an observation made upon one of the tailed Amphibians by Schectman ('34). This investigator stained the vegetal pole of a fertilized *Triturus* egg and found that by the midblastula stage the stain occupied cells some distance from the surface, and near to the floor of the blastocoel. He did not follow the material in later stages, and refers to its inward movement as "unipolar ingression." This he properly enough indicates as occurring during "blastulation" (cleavage), and does not suggest that it has anything to do with gastrulation. However, he does note that it seems to be involved in the upward movement of the blastocoel floor, in this case at its middle, and this movement, it may be recalled, is one which we have designated as a part of modified invagination. Whether, therefore, this movement in *Triturus* is really to be regarded as a kind of premature and greatly modified invagination, and hence a precocious aspect of gastrulation, is a question for further study. At least it is a possibility to be borne in mind. Finally it may be added that the process in question acquires additional

As has been suggested this internal process is going on simultaneously with the externally observable process of epiboly. As a result of both a new cavity is being formed which replaces the blastocoel. It is the *archenteron*, and is lined by hypoblast, a relatively thin layer forming the roof and the main mass of yolk-filled cells constituting the floor.

Involution. — It now remains to point out that in addition to the processes so far described there is also a distinct process of involution. This is most active at the median dorsal lip and progressively less so as one passes around either side, until at the ventral lip there is almost none at all. The immediate cause of this movement, as well as of such invagination as occurs, is apparently a change in shape of the cells adjacent to the lips and in the yolk plug.

From this account, the roof and sides of the archenteric interior consist of material originally outside, dorsal and lateral to the blastoporal lip, while the floor is composed of cells originally on the outside of the vegetal region. The latter seem to have moved into their definitive position by an inpushing and inturning of the yolk cells called modified invagination (Fig. 68). Any inwandering of individual vegetal cells (*ingression*), as implied by Schectman and others (see footnote) is denied by Ballard, '55, who says that only the stain moves in, no cells.

Delamination. — In the general account of gastrulation in Chapter II, it will be recalled that the origin of endoderm by the process of splitting off was said to occur to a slight extent among the Amphibia. It should here be stated, however, that its occurrence is not universally admitted. Those who do describe it (Brachet, for instance) say it takes place in the following manner:

Reference to the figures will indicate that, as the process of invagination begins, one of the results is as follows: As the yolk cells (hypoblast) about the margins of the blastocoel are pushed upward, they tend, as previously noted, to obliterate the portions of this cavity between themselves and the epiblast. The obliteration, however, is not quite complete, so that between the uprising hypoblast and the epiblast there remains a slight crevice. The upward extent of this crevice is then obviously increased by the continuance of the above processes. By those who maintain the existence of delamination, however, it is held that

interest in the light of Peter's observations on the inwandering of cells in the gastrulation of the Chick (see gastrulation in the Chick). In that case, however, the movement is into the blastocoel from a layer over it instead of from the yolk beneath it. Perhaps, however, in view of the changed relationships in the Bird, due to excess yolk, this difference is not significant.

besides this upward extension there is also a well marked downward extension, i.e., in the direction of the blastoporal lips. This appears to occur first, but least extensively, in the margin of the blastocoel nearest the dorsal lip, whence it presently extends entirely around the circumference and becomes most extensive toward the ventral lip. Here it apparently serves throughout a considerable region to separate the yolk-filled cells from the epiblast on the definitive ventral side of the gastrula. The significant point, however, is the fact that wherever the process takes place it is due apparently to a splitting apart or delamination of the cells at the bottom of the crevice (Fig. 68, *gc*). But since at all points this crevice serves to separate epiblast from hypoblast, its downward extension in the manner indicated is obviously setting apart these layers by delamination. In this particular situation this separation has also been given the name of *gastrular cleavage*.

Summary of the Processes. — To sum up the processes involved in the gastrulation of the Frog, it is found that there are four of them which also occurred in *Amphioxus*, i.e., epiboly, involution, invagination and convergence or confluence. In addition there seems to be some delamination which appears here for the first time. Though a common method for setting aside mesoderm and notochord, it is not so commonly thought of in connection with gastrulation. As we shall see, however, it is perhaps the only method in Mammals, and possibly also in Birds.

In connection with these gastrulation processes it may finally be noted that there has been a considerable shifting of the yolk mass, and hence of the center of gravity. It is to these shiftings, apparently, that the rotation of the gastrula is due.

MESODERM, NOTOCHORD AND NEURAL PLATE

The Mesoderm and the Notochord. — As the archenteron develops the layer which is invaginated, involuted, or delaminated to form its roof has been referred to as hypoblast. It now appears that this hypoblast contains the elements of a part of the endoderm, and all of the mesoderm, including the notochord. The setting aside of these layers occurs as the result of a delamination from the hypoblast. The lower layer of cells thus split off forms the *endoderm* of the archenteric roof and sides. It is of course continuous ventrally with the yolk cells which become the endoderm of the floor. The upper layer resulting from this split lies between the newly formed endoderm of the roof and sides and the epiblast. This in between layer is *mesoderm*, while the overlying epiblast may now be called *ectoderm*.

It should here be noted that the splitting off of the mesoderm does not occur everywhere simultaneously, but begins on either side and proceeds toward the median line. Here for a time a narrow strip of cells remains connected with the underlying layer. Presently it is separated both from the endoderm beneath and from the mesoderm on either side. It is the *notochord* (Fig. 69). The mesoderm of the ventral part of the embryo is formed later mainly by a downgrowth of the lateral sheets between the endodermal yolk mass and the ectoderm. Anteriorly it occurs not as a definite layer, but rather as loosely arranged cells, a type of mesoderm generally referred to as *mesenchyme*.

Presently by the above means the mesoderm comes to exist throughout the greater part of the embryo, as a separate layer between ectoderm and endoderm. As noted, it is interrupted dorsally by the notochord, while anteriorly the cells are very loosely arranged.

Lastly in the region of the blastopore there persists for a time an undifferentiated mass of cells containing the elements of all three layers. These gradually become defined, as the blastopore closes.

The Medullary or Neural Plate and Related Structures.— It has already been noted that at the end of segmentation the epiblast of the animal hemisphere was split into an outer layer and an inner nervous layer. During gastrulation this becomes true also in the vegetal hemisphere. Thus toward the latter part of that process, a double layer of ectoderm exists everywhere except in the immediate vicinity of the blastoporal lips. Throughout certain regions of the gastrula the nervous

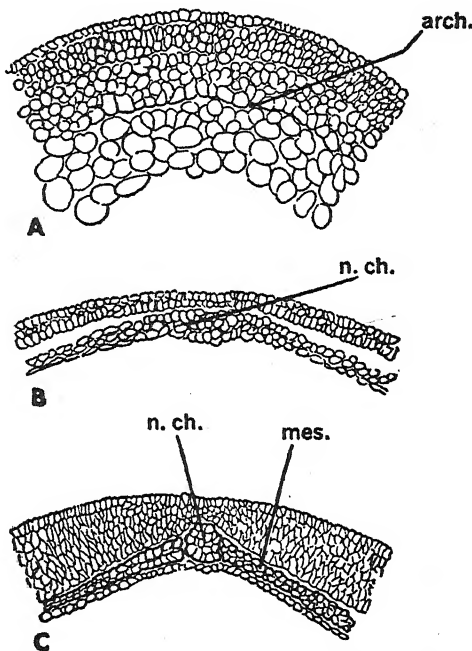
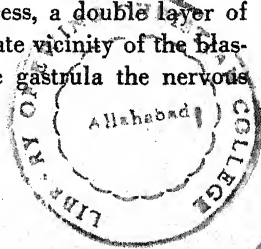


Fig. 69.—Three stages in the differentiation of the roof of the archenteron in the Frog. From Jenkinson (*Vertebrate Embryology*).
arch. Archenteron. n.ch. Notochord. mes. Dorsal Mesoderm.



ectodermal layer then begins to thicken, the thickening being defined as the *medullary* or *neural plate*. This plate extends forward from the dorsal blastoporal lips as a median band, widening rapidly as it approaches the anterior end of the gastrula. Here it terminates, the extremity having the form of a broad curve (Fig. 77, *A*).

The thickening process which has given rise to the plate presently grows most marked around its margins, and these become slightly elevated. The elevations which thus occur along the sides of the plate are the beginnings of the *lateral neural ridges* or *folds*, while around the anterior end they are continuous with one another as the *transverse neural ridge* or *fold* (Fig. 77, *B*). Accompanying or immediately following the thickening of the nervous ectoderm which produces the ridges, there is a corresponding thinning of this layer along the mid-line of the plate. As a result there soon appears here a shallow depression. It is sometimes scarcely evident externally at this stage, but as soon as it becomes so, it is termed the *neural groove*.

EXPERIMENTAL RESULTS

Some of the most significant work in modern experimental embryology has been done upon the early stages of Amphibian development. There have been two main lines of investigation. One has interested itself in the movements and fate of materials during gastrulation, while the other has sought information concerning the effect of these materials upon one another. Though the aims of these studies have thus been somewhat different, the results, as will presently appear, have largely tended to supplement each other.

Location and Movement of Materials During Gastrulation. — One important method for discovering the movements and fate of materials during this process has been to stain the surface of very early gastrulae with vital stains at certain significant points, and then observe the shifts in these stains in later development. This is possible in some of the Urodeles, such as Triton, which possess unpigmented eggs, and has been done by Vogt, Goertler, and others, with the external results shown in Fig. 70. Other experiments, noted presently, help prove the reality of the involution of part of this material, as already described, to form the hypoblast of the roof and sides of the archenteron. On the basis of these results Vogt and Goertler constructed more or less idealized maps showing their views as to the location of this hypoblast (potential endoderm, mesoderm and notochord) previous to gastrulation. Their conclusions are shown in Figure 71.

More recently the matter has been reinvestigated by Pasteels ('42) in another Urodele, *Axolotl*, and in an Anuran, the primitive Frog, *Discoglossus*, the results being indicated in Figure 72. It will be noted that aside from differences between the older and newer maps of the Urodeles there are also some differences between those of the Urodeles and the Anurans. On the whole, however, these are matters of detail, the fundamental patterns being similar in all of them.

Aside from these minor differences shown in the pregastrula maps, there is one alleged post-gastrula difference between the Urodeles and at least most Anura which the maps partly suggest but do not really show, and which is perhaps worth mentioning. It has to do with the

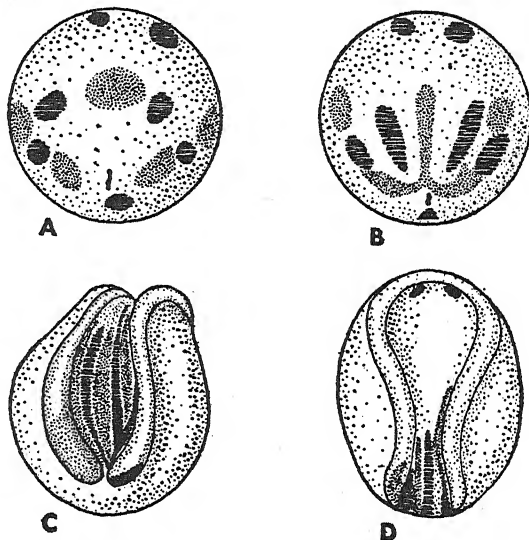


Fig. 70. — Four stages in the development of a Triton egg which had been marked with dyes in the early gastrula stage. The changes in shape and position of the colored areas indicate the movements of the materials of the egg during gastrulation and the formation of the medullary folds. After Goertler.

A. The early gastrula from the postero-dorsal side. B. A slightly later stage from the same view point. C. A much later stage viewed from the posterior. The neural folds are in evidence, but the blastopore does not show. D. About the same stage as C viewed from the dorsal side.

actual setting aside of notochord and somitic and lateral-plate mesoderm from endoderm, and is as follows: We have already noted that in the common Frog, *Rana*, the materials for the notochord, somites, dorso-lateral mesoderm and endoderm are involuted as a single sheet of hypoblast. This hypoblast is then later separated by delamination into notochordal, somitic and lateral-plate mesodermal material above, and the endoderm of the archenteric roof beneath. In the Urodeles, however, this is not true. The involuted hypoblastic roof of the archenteron turns out to be composed exclusively of the definitive notochord and somites with perhaps even a little of the dorso-lateral mesoderm. This roof thus lacks

temporarily any endoderm; the latter being presently supplied, not by delamination, but by the upgrowth of endoderm lying lower down on either side.

In concluding this topic there is this further point to note: The external area indicated by these maps as giving rise to the notochord and at least parts of the mesoderm and endoderm is also approximately the area of the gray crescent in those cases where there is one.

The Region of the Gray Crescent as the Center of Organization. — Turning now to the problem of how the materials affect one

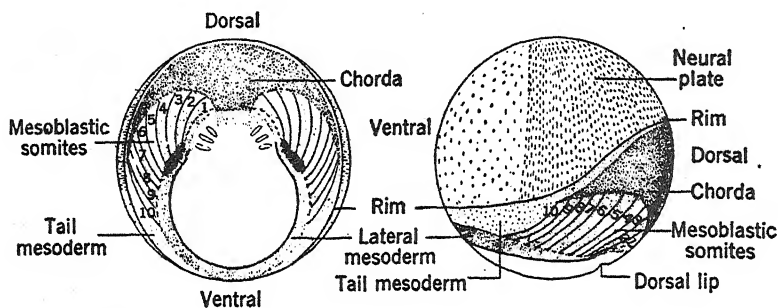


Fig. 71. — Diagrams of a *Triton* egg previous to gastrulation, showing the supposed location of the materials which, with the exception of the neural plate, are destined to be involuted to form various structures as indicated. *A*. View from the vegetal pole. *B*. Side view. After Vogt.

Rim. The region which eventually becomes the lip of the blastopore at the end of gastrulation.

another a long series of experiments might be cited. Only enough will be mentioned, however, to indicate what the trend has been, and the important conclusions which have at present been reached.

As has already been made clear, though the first cleavage in the Frog tends to bisect the dorsal lip of the blastopore, it does not always do so. In some cases indeed it may come as far as possible from this, and lie parallel to this lip. Brachet ('05, '06) took advantage of this fact to find out what would happen when one of the blastomeres of such an egg was killed, as had previously been done with the blastomeres of more normal cleavages. In the latter case it will be recalled one side of an embryo developed, unless the egg had been so treated as to rearrange materials related to the gray crescent. In the latter event a whole, or nearly a whole, embryo was formed. Now in Brachet's eggs it is clear that the crescent will be in only one of the two hemispheres, i.e., the one containing the dorsal blastoporal lip. It is perhaps not surprising therefore that when one blastomere of such an egg was killed, the re-

maintaining one would only develop when it was the one which contained the crescent. These, moreover, formed better than half of the anterior and dorsal part of an embryo. Thus once again the importance of materials connected with the gray crescent region was demonstrated (Fig. 73).

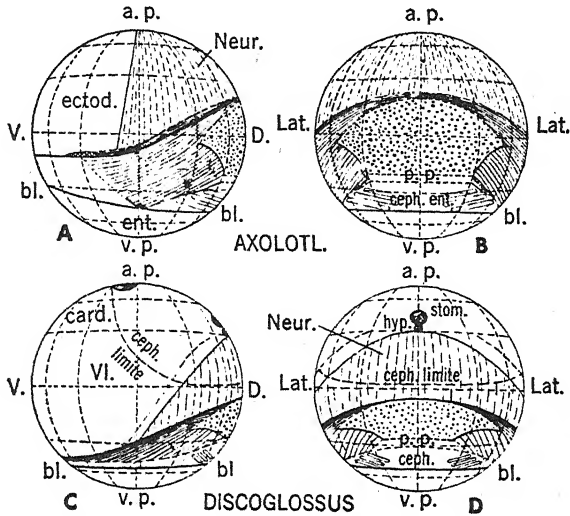


Fig. 72. — Maps of young gastrulas of (A and B) Axolotl, a Urodele, and (C and D) Discoglossus, an Anuran, showing the location of materials destined for various structures. After Pasteels. The figures to the left (A and C) show the gastrulas from the left side, while the figures to the right (B and D) show them from the future dorsal side.

D. Dorsal. V. Ventral. Lat. Lateral. bl. Blastopore. a.p. Animal pole. v.p. Vegetal pole. Stippled areas, notochord. Vertically-hatched areas, neural ectoderm. Diagonally-crossed hatched areas, mesoderm. Clear areas toward bottom of page from the mesoderm are endoderm. They contain bars representing material for the future gill slits.

The next step was taken by Spemann and Mangold ('24). These men took a small piece of material just anterior to the dorsal lip of a Triton early gastrula and grafted it upon the surface of another gastrula. Wherever it was placed, this material was soon covered over by surrounding cells, and the cells which covered it presently formed a medullary plate. Later this plate would give rise to a neural tube, or part of one, as shown in Figures 74, 75. The same experiment was eventually done with the Frog. Also Bautzmann ('26) performed more detailed experiments to see how far from the blastoporal lip of an early gastrula

the material possessed this power to cause other ectoderm to become neural plate. She found the region extended about 85 degrees anterior to the middle of the lip, and about 80 degrees to either side, the anterior extent decreasing as one proceeds laterally. The effective area thus had the form of a crescent occupying a similar but somewhat wider zone than that occupied by the gray crescent when the latter exists.

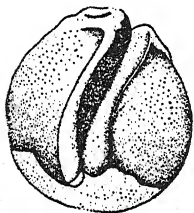


Fig. 73.—A Frog embryo produced by injuring one of the first two blastomeres in a case where the first cleavage plane was parallel to the gray crescent instead of being at right angles to it, as is usual. The blastomere injured was the one which did not contain the crescent, since otherwise no development occurs. The uninjured cell has developed into somewhat more than half of the anterior portion of an embryo. After Brachet.

Now normally of course the material transplanted in these experiments reaches a position beneath the ectoderm by being involuted over the lip of the blastopore. Hence involuted material (hypoblast) taken from archenteric roof of a late gastrula should also be expected to stimulate neural plate formation in any ectoderm under which it occurs. Marx ('25) and Geinitz ('25) tested this assumption by transplanting such involuted hypoblast beneath other ectoderm than that which normally produces neural plate. The assumption proved correct (Fig. 76). Indeed this fact is one of the proofs that involution occurs.

Though the action of potential chorda mesoderm in inducing neural tube formation has thus been proven, another question still remains. Is all the ectoderm of a blastula or early gastrula really entirely equivalent in its potentialities? Though chorda mesoderm will induce neural tube formation in any ectoderm is it not possible that some ectoderm, namely that of the normal neural plate region, might form neural tube without any chorda mesoderm present? Attempts to answer this question have been made by several workers, notably Spemann ('18, '21). This worker transplanted small pieces of ectoderm from the prospective neural plate region of a young gastrula to a different region in another gastrula. He also performed the converse experiment of placing ordinary ectoderm in the position of part of the prospective neural plate. In some of the cases, moreover, he made the interchange between different species of Triton having ectoderm of distinctly different shades. Thus it was possible to follow accurately the fate of the transplants in their new environments. The results in all cases showed that at this stage of development the fate of the ectoderm has not yet been determined. The prospective neural plate material when placed

elsewhere did not form neural plate, but ectoderm like that surrounding it in its new location, while the latter when implanted in the midst of the future neural plate became a normal part of the plate and future neural tube. Later work by Marx ('25), it is true, showed that just before the neural plate appears the ectoderm has become determined, but previous to that time the results are as indicated. These data therefore would seem to prove that in very early gastrulae the ectoderm of the prospective neural plate region has not yet come under the influence of the chorda mesoderm, and that under these circumstances it has the same, or nearly the same, potentialities as in any other location (see below).

The Principal of Induction. — The action of a substance in thus causing cells to respond by forming some specific tissue or structure is known as *induction* or *evocation*. The tissue which responds, on the other hand, is said to have a certain *competence*. Although such a relationship has received its greatest emphasis in connection with the material in the vicinity of the dorsal lip of the Amphibian blastopore, this particular instance is by no means unique. It occurs in many organisms, and in connection with all sorts of tissues and stages of development, some of the more striking examples of which will be pointed out as we come to them. Because of the early discovery and far reaching consequences of the inducing material in the vicinity of the Amphibian blastoporal lip, however, it was especially designated as the *organizer*.

It must now further be added that the induction and response relationship in general is not always such a completely open and shut one as so far indicated. Some tissues have different degrees of inducing capacities, while the competence of other tissues to respond in a specific way varies considerably as one proceeds away from the site where a particular response normally occurs. Thus even in the original case of neural plate induction, it now appears possible that, contrary to some of the earlier results indicated, not all ectoderm is quite alike in its ability to respond. Some areas of ectoderm form neural plate and tube more easily than others, especially if properly oriented (Barth, '41). It should also be stated that when a tissue has once begun to respond in a certain direction, it loses its competence to respond in any other.

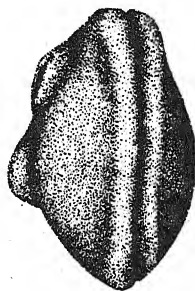


Fig. 74. — An embryo of Triton on whose left side an extra neural tube has been induced. This was done by implanting in the side of this embryo at the gastrula stage a piece of external material from the blastoporal lip of another gastrula. After Spemann and Mangold.

The Nature of the Inducing Substance.— It now remains to add a word regarding more recent attempts to analyze the nature of inducing substances, particularly the original one designated as the organizer. The first steps in this direction involved efforts at discovering how specific the inducing substance was, i.e., would anything other than material related to the gray crescent region induce neural tube?

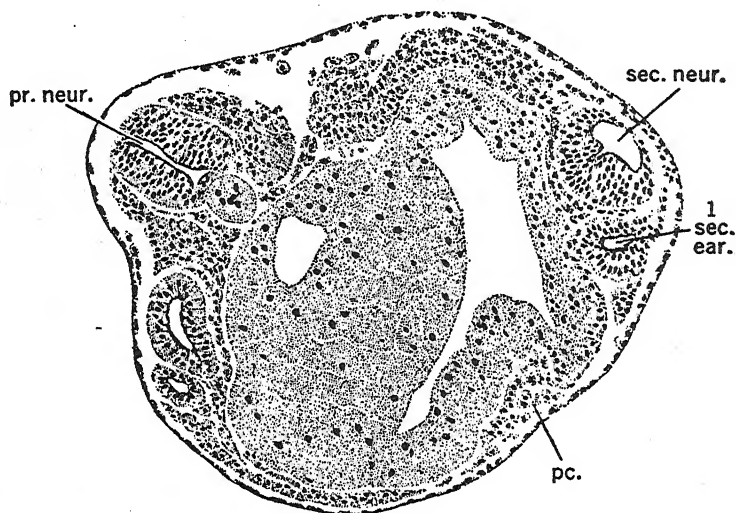


Fig. 75.— A cross section of the same embryo shown in Fig. 74, at a later stage, showing the two neural tubes. After Spemann and Mangold.

l. sec. ear. Left secondary ear vesicle. *pc.* Pericardial cavity. *pr. neur.* Primary neural tube. *sec. neur.* Secondary or induced neural tube which because of the orientation of the section appears on the right instead of the left side.

The answer was rather startling. It was found that a very wide variety of materials would work, e.g., pieces of adult liver and kidney as well as certain Invertebrate tissues like ganglia of *Lepidoptera*. It has further been discovered that a tissue which normally lacks inductive capacity, such as neural plate, may acquire it by being in contact with one which normally possesses it, such as chorda-mesoderm. Indeed it is now known that neural tube, having itself been induced, is then capable for a time of inducing tube formation in undetermined ectoderm. It was also shown that tissues need not be alive or recently killed. Tissues would work even after being fixed and imbedded in paraffin as for sectioning. Not only this but in some instances material such as pieces of blastula which normally have no inducing capacity will act as inducers after they have been boiled! Thus it is clear that the substance

concerned is non-living, and is fairly widespread. From this point it would seem that with modern analytical methods it should not be too difficult to trace down the essential chemical involved. Such, however, has proved far from the case. Many workers have attacked the problem, among the most prominent being Spemann in Europe, Needham and Waddington in England and Holtfreter and Barth in the United States. Spemann believed that glycogen might be the substance, but Needham thinks that one of the sterols is responsible. Barth and Graff

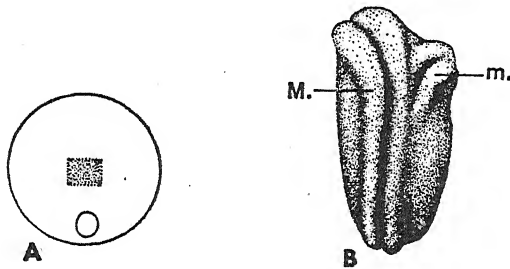


Fig. 76. — *A*. Diagram of Bombinator (a Toad). The small circle indicates the blastopore, and the shaded square represents the region from beneath which a piece of the archenteric roof was taken, and transplanted to the blastocoel of a gastrula of Triton. *B*. An older stage of the Triton to which the transplant from (*A*) was made. After Geinitz. *M*. The regular primary neural tube. *m*. The partial secondary tube induced by the transplant.

('38), on the other hand, doubt the possibility of determining with certainty just what the normally acting material may be. The difficulty is that various chemicals and treatments, some of which are probably actually toxic, nevertheless have an inductive effect. It seems unlikely that so many different substances are concerned under natural conditions, and it is certainly unlikely that any of them are toxic. It has been said that these chemicals are not the inductors, but release the latter from the live tissue. Also it is possible that the process may consist of the removal of a blocking substance which has inhibited various developmental possibilities inherent in the cells acted upon. Finally, the reason for different reactions by like material, e.g., the formation from the neural plate of brain in one place and neural tube in another, may be due either to a quantitative or qualitative difference in the inductor produced by different regions of the archenteric roof (Barth, '53).

Significance of Developmental Concepts. — In concluding this general topic it is well to emphasize first the very great importance of the

fundamental concept of induction. As this concept becomes increasingly established and elaborated we can see, at least theoretically, how a complex structure like an embryo may develop from a specific physico-chemical system like an egg. Thus, when the equilibrium of this system is disturbed by fertilization or otherwise, an orderly chain of reactions is started, each one inducing others. Obviously this does not completely explain development. Yet it does reveal a significant aspect of it which will be repeatedly demonstrated as we proceed.

Recently Townes and Holtfreter, '55, have discovered something which may help to establish another concept. By mixing ectoderm, mesoderm, and endoderm cells from neuralae-gastrulae they have shown that these cells possess certain "directive movements and selective adhesiveness" characteristic of each cell type, causing some to move inward, while others spread peripherally, arranging themselves in normal tissue patterns.

A COMPARISON OF GASTRULATION, MESODERM AND MEDULLARY PLATE FORMATION IN AMPHIOXUS AND THE FROG

A comparison of gastrulation, mesoderm and notochord formation, and the development of the medullary plate in *Amphioxus* and the Frog may now be presented in tabular form, as follows:

Gastrulation

AMPHIOXUS

The processes involved are: invagination, involution, epiboly, and convergence.

FROG

The processes involved are: modified invagination, involution, epiboly, some convergence, and delamination.

Mesoderm Formation

1. Gastrulation is virtually completed before definite setting aside of mesoderm begins.

2. The potential mesodermal material is identifiable in the fertilized egg. It can be traced into the ventro-lateral blastoporal lip of the early gastrula, whence it is carried into its definitive position

1. Gastrulation is completed before mesoderm is set aside.

2. The potential mesodermal material is not visually distinguishable until after gastrulation, but evidence shows that it exists lateral to the lips of the blastopore. Thence it is brought into its defini-

AMPHIOXUS

by a kind of combined involution, epiboly, and convergence.

3. The setting aside of the mesoderm in the form of somites occurs by a process closely akin to enterocoelic evagination, especially in the more anterior region.

FROG

tive position by processes of involution, epiboly, and convergence.

3. The dorsal and lateral mesoderm is set apart as such by delamination. Ventrally, however, it arises to a considerable extent by the proliferation of cells from that already formed.

The Notochord

The potential notochordal material occurs at the dorsal lip of the blastopore. Thence it is involuted to the archenteric roof from which it is set aside by evagination.

The potential notochordal material lies anterior to the dorsal lip of the blastopore. Thence it is involuted to the archenteric roof. From this roof and from the mesoderm on either side it is then separated by delamination.

The Medullary Plate and Folds

1. There is no split between outer and nervous ectoderm. Dorsally a median strip of ectoderm becomes slightly depressed to constitute the medullary plate. The edges of the ectoderm on each side of this plate presently become separated from the margins of the latter, and then grow together above it. The overgrowing layers so formed thus constitute only the outer half of a true medullary fold. *Later*, the margins of the plate itself also bend toward one another until they meet and fuse beneath the overgrown ectoderm.

2. In *Amphioxus* no attempt has been made to demonstrate induc-

1. An inner or nervous layer of ectoderm is formed by delamination over the entire gastrula. The medullary plate arises by a thickening of this layer in the mid-dorsal region. As will appear below, the margins of this plate then come to constitute the crests of true neural folds. This follows from the fact that in this case the sides of the plate are carried upward and together, not *later* than, but in *company with* the ectoderm around their edges. Thus no separation occurs between the ectoderm of the plate and that surrounding it until the crests of the folds meet.

2. In the Frog experimental procedure has demonstrated that

AMPHIOXUS

tive action. However, it very probably occurs here as in the Amphibians and other forms.

FROG

the ectoderm is stimulated to form neural plate and tube by the inductive action of the underlying chordo-mesoderm.

In concluding this comparison it is well once more to emphasize the fact that the above differences, at least those of gastrulation and mesoderm formation, are chiefly due to differences in relative amount of yolk. It may also be repeated that a further increase in this substance in the Fish and Bird is apparently responsible for the still greater modifications of the above processes in those animals.



THE FROG: EARLY OR EMBRYONIC DEVELOPMENT SUBSEQUENT TO GASTRULATION

THE general condition of the embryo at the conclusion of gastrulation has already been indicated, and there was also noted the origin of the notochord, the mesoderm, the medullary plate and neural folds. Following this there occurs a period characterized by the beginning of elongation and also by the appearance of the rudiments of the main systems and organs. Thus at the end of the time in question, during which the animal has reached a length of from 2.5 to 3 mm., virtually all these rudiments are present. For this reason it will be convenient to carry forward the description of both external and internal development to about this point. We shall then be prepared to describe more clearly the remaining changes which lead to the formation of the adult.

In carrying out this plan it will not be possible to state with any accuracy the age at which a particular size and degree of development is reached, even in the same species of Frog. This is necessarily so on account of the variableness of temperature to which the eggs are subjected. It will nevertheless be helpful occasionally to mention the average age of embryos of a given condition. The student must clearly bear in mind, however, that this is never more than approximate. It is desirable to begin by considering the development of this early period in its external aspects.

EXTERNAL CHANGES

As the embryo begins to elongate certain rather conspicuous features arise as elevations or depressions of the surface. All of these structures are at first more or less connected with the medullary plate, and all of them appear at about the same time. It will be necessary, however, to describe them separately.

External Development of the Neural Tube.—The neural groove whose beginning has been noted, now becomes much deeper and more prominent (Fig. 77, C). At the same time the lateral neural ridges or folds begin to increase their elevation and to bend toward one an-

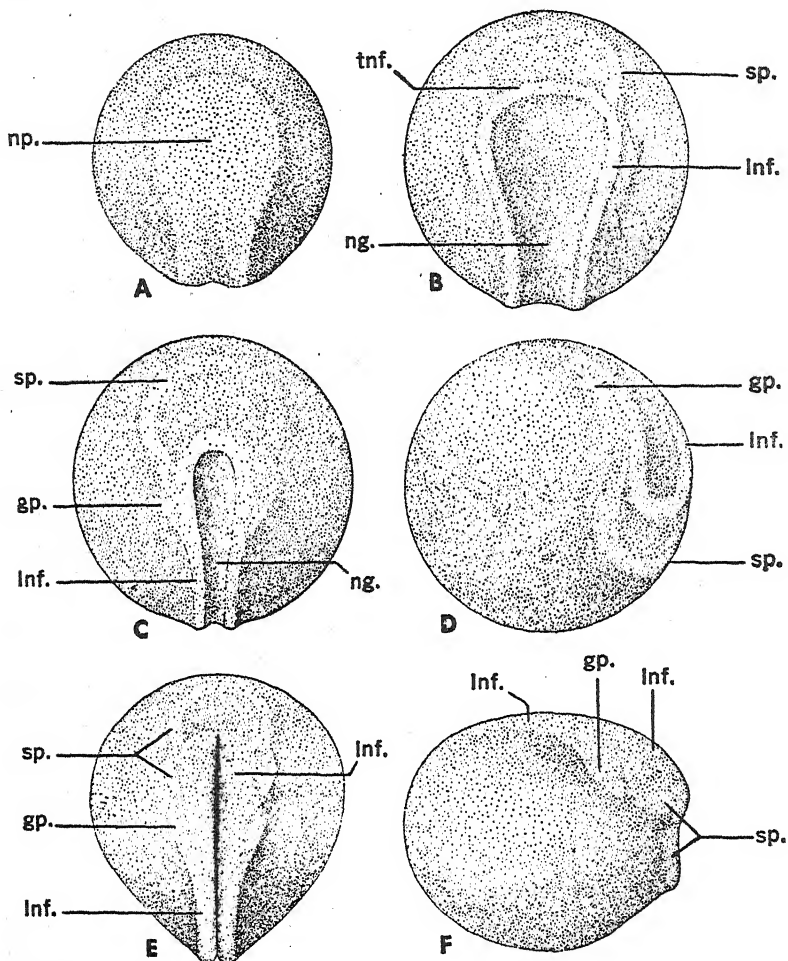


Fig. 77.—Drawings of preserved Frog embryos (*Rana pipiens*) showing successive stages in the development of the neural tube, the sense plate and the gill plates. *A*. Antero-dorsal view of a stage shortly after the completion of gastrulation, showing the neural or medullary plate. *B*. Same view of the next stage, showing the beginnings of the neural folds and the sense plate. *C*. Same view of somewhat later stage, showing the beginnings of the gill plates. *D*. Antero-lateral view of same specimen. *E*. Antero-dorsal view of still later stage, showing neural folds about to fuse. The sense plate and gill plates are clearly marked. *F*. Lateral view of same specimen.

gp. Gill plate. *Inf.* Lateral neural, or medullary folds. *ng.* Neural groove. *np.* Neural, or medullary plate. *sp.* Sense plate. *tnf.* Transverse neural or medullary fold.

other until eventually their crests meet and fuse; thus is formed the neural tube. Further, as noted above, the neural plate in this case, as in that of all true Vertebrates, is involved in the process from the first. Hence no break occurs along the crests of the folds between their outer and inner layers until after these crests have met (Fig. 80) ¹ The phenomenon thus indicated starts somewhat anterior to the middle of the embryo in about the region of the future medulla, and from here the fusion proceeds in both directions. Anteriorly, this lateral closure is further augmented by the back growth of the transverse neural fold. Nevertheless, as will be noted presently, the completion of the process occurs later in the anterior region because of the greater space which separates the folds in this vicinity. The tube which is thus formed soon appears as a prominent ridge along the back.

The Sense Plate and the Gill Plate. — During the above process there are also developed certain other structures as follows: Just as the medullary ridges are preparing to fold in, a slight and rather narrow elevation grows outward from the antero-lateral region of each of them, and begins to extend in an antero-ventral direction. This continues until the two elevations meet one another on the front of the embryo some distance below the anterior edge of the transverse neural fold (Fig. 77, *B*). There is thus formed a relatively narrow band of slightly elevated tissue which traverses the lower anterior region of the embryo in a broad curve and then ascends on either side until it merges with the edges of the neural folds. It is termed the *sense plate*. For a time the median area between the inner edge of this semicircular band-like plate and the edge of the transverse neural fold above it remains relatively depressed; i.e., of no greater elevation than the region outside the plate. Presently, however, the distinction between this median area and the plate which constitutes its ventral and lateral boundary gradually lessens, the central region becoming almost as much elevated as its border. In this manner the sense plate comes to constitute a broad, somewhat shield-shaped region extending across the front of the embryo from side to side, while dorsally it is more or less continuous with the anterior of the neural tube (Fig. 77, *E, F*).

During the course of these processes another event is taking place immediately posterior to those portions of the sense plate where it joins the neural folds upon either side. In each of these two regions there is

¹ It is to be noted that these lateral crests of the folds are not quite identical with the "neural crests" referred to below in Fig. 80, the distinction becoming clear as the tube is about to be completed.

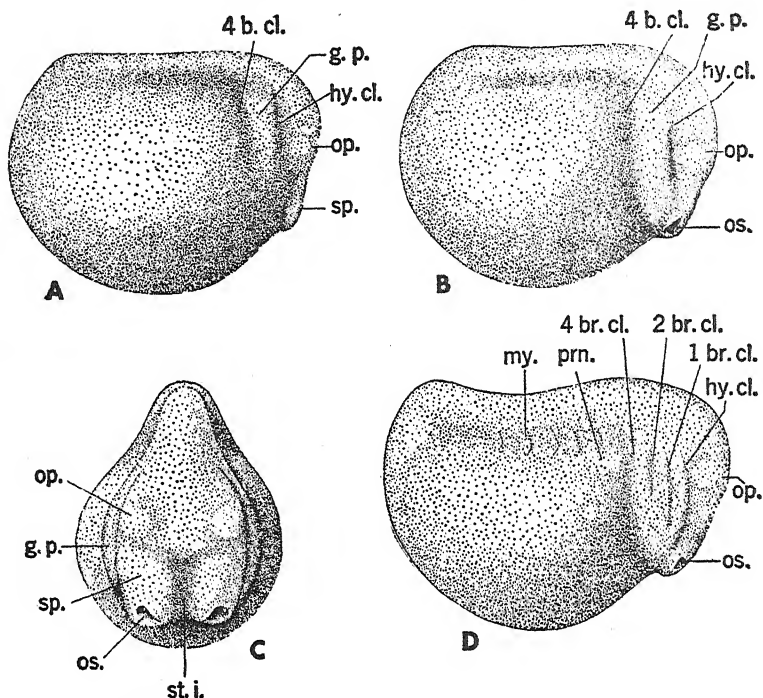


Fig. 78. — Drawings of preserved Frog embryos (*Rana pipiens*) from 2.2 to 2.5 mm. in length, showing particularly the changes in the sense and gill plates. *A.* Right side of a 2.2 mm. embryo. The outpushing of the optic vesicle is just beginning to appear on the dorsal part of the sense plate. The latter is becoming more clearly separated from the gill plate by the rudiment of the hyomandibular cleft, while the posterior boundary of the gill plate, i.e., the rudiment of the fourth branchial cleft, is also becoming more evident. *B.* Right side of a slightly older embryo than *A.* The invagination of the left oral "sucker" (mucous gland) is visible near the ventral end of the sense plate. *C.* The same embryo viewed directly from the anterior end. The stomodaeal invagination and the two parts of the developing mucous gland are clearly shown. *D.* A 2.5 mm. embryo from the right side. The rudiments of the first and second branchial clefts have appeared upon the gill plate. Also, just posterior to the dorsal part of the gill plate the outpushing due to the pronephros is visible, and the external indications of some of the myotomes are beginning to appear.

1br. cl. *2br. cl.* *4br. cl.* Rudiments of the first, second, and fourth branchial (gill) clefts. The arch anterior to each cleft is named in the text. *gp.* Gill plate. *hy.cl.* Rudiment of hyomandibular cleft. *my.* External indication of one of the myotomes. *op.* External indication of the outpushing optic vesicle in the upper region of the sense plate. *os.* Rudiment of oral "sucker" or mucous gland in the lower region of the sense plate. *prn.* External indication of the pronephros. *sp.* The sense plate, whose lower portion really represents the mandibular arch. *st.i.* The stomodaeal invagination.

developing another elevation which extends outward from the neural folds approximately parallel with the posterior border of the sense plate. Indeed, each of the new elevations is said by some authors to be merely a part of the original plate separated from it by the development of a depression. In any event the new raised areas, because of their future development, are termed *gill plates* (Fig. 77, C, D, E, F).

As the anterior portions of the neural ridges meet one another, a slight protuberance arises upon either side of the dorsal region of the sense plate (Fig. 78, A). These protuberances mark the outpushings of the two optic vesicles (see below). Also at about this time there begins to develop in the middle of the sense plate a rather wide vertical groove extending from near its ventral margin dorsally to about the level of the lower edges of the optic protuberances (Fig. 78, C). This is the *stomodaeal invagination*, the stomodaeum proper, forming later at its dorsal end. It is evident that the development of this groove results in a division of the sense plate throughout the greater part of its length, so that the raised portions exist only upon either side of the median line. It may now be added that each of these raised areas constitutes the rudiment of one side of the future lower jaw or mandible, and hence each such area is designated at this time as a *mandibular arch*. Lastly, at the ventral end of each of these arches there now develops a small, somewhat elongated, and slightly pigmented depression. These depressions then deepen, while their postero-ventral ends grow toward one another and fuse, thus forming the characteristic V shaped "sucker" or *mucous gland* of the early larva.

It has been noted that the sense plate (now represented by the mandibular arches) is separated from each gill plate by a slight furrow; it remains to be added that a similar indentation also bounds each of the latter plates posteriorly (Fig. 78). Upon either side the more anterior of these furrows, i.e., the one between the mandibular arch and gill plate, marks the location of the *hyomandibular "cleft"* (in this case never an actual cleft), while the posterior one indicates the approximate position of the future *fourth branchial (gill) cleft*. There next appear upon the surface of each gill plate itself two more vertically elongated depressions denoting the beginnings of the *first* and *second branchial clefts*, the rudiment of the third branchial cleft not developing until somewhat later (Fig. 78, D).

It is now further obvious that, between the depressions just noted, the surface of each gill plate will be relatively raised so as to form ridges which are the external indications of the hyoid and branchial

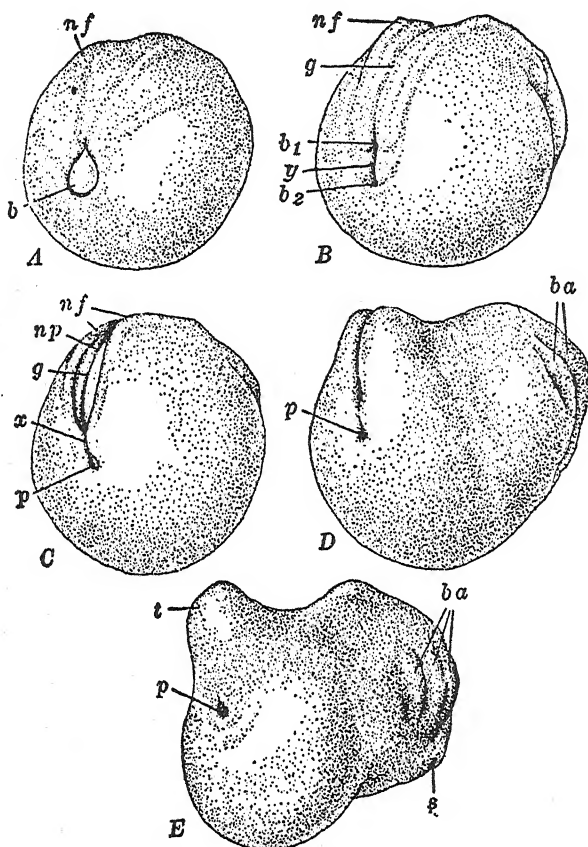


Fig. 79. — Posterior ends of a series of young Frog embryos, showing the later history of the blastopore, and the relation of the neural folds to it. The embryos are viewed obliquely from the postero-lateral aspect. From Kellicott (*Chordate Development*). After F. Ziegler. A. Blastopore nearly closed, neural folds just indicated. B. Blastopore becoming divided into neurenteric and proctodaeal portions, lips between fusing to form primitive streak; neural folds becoming elevated. C. Neurenteric canal forming; neural folds closing together. D. Neural folds in contact throughout. E. Neural folds completely fused; tail commencing to grow out.

b. Blastopore, containing yolk plug. b_1 . Rudiment of neurenteric canal (dorsal part of blastopore). b_2 . Rudiment of proctodaeal pit (ventral part of blastopore). ba. Branchial arches. g. Neural groove. nf. Neural folds. np. Neural plate. p. Proctodaeal pit. s. Rudiment of oral "sucker." t. Rudiment of tail. x. Neural folds roofing the blastopore and establishing the neurenteric canal. y. Primitive streak.

arches. The most anterior portion of the plate which lies between the hyomandibular cleft and the first branchial cleft is the *hyoid arch*, while the portion lying between the first and second branchial clefts is the *first branchial (gill) arch*. Since the third branchial cleft has not yet appeared, the portion of the plate posterior to the second branchial cleft really represents both the second and the third branchial arches.

The Closure of the Blastopore. — As the above events are transpiring anteriorly, certain processes are also occurring posteriorly, as follows: As the medullary folds begin to move toward one another, the lateral lips of the blastopore also draw together, so that the latter is no longer round. Instead it has the form of a short vertical slit (Figs. 79, *B* and 80). Presently, moreover, these lips fuse with one another for a certain distance midway between their dorsal and ventral ends. As a result there may appear in this region for a time a slight vertical groove connecting the dorsal and ventral openings which temporarily remain. In the present case this is the *primitive streak*. In it, ectoderm, mesoderm, and endoderm meet in one mass, and from this mass, cells for all three layers are budded as the embryo increases in length. It is very important to note that this primitive streak is homologous with the similarly named structures which are to be described in connection with the next two forms. It is also probably comparable with the primitive streak of Birds and Mammals, and with the structure similarly defined in the general discussion in Chapter II. This question will be discussed more fully in connection with the Chick.

The opening which remained at the ventral lip closes presently, but only the ectoderm and endoderm are involved. Hence the wall is thin at this point, and a slight pit remains. It is the *proctodaeum* (Figs. 79, *D* and 80). The dorsal opening of the blastopore persists for a somewhat longer time. It disappears externally, however, because the neural folds which extend on either side of it fuse at this point as elsewhere, and thus roof it over. This process will be further noted in connection with the nervous system.

Other Changes. — Besides the features already mentioned there are a few other external alterations which usually become apparent by the time the embryo is from 2.5 to 3 mm. in length. In the first place, in connection with its slight elongation, the animal has begun to lose its spherical form, so that the convexly curved line of the back (Fig. 77, *F*) becomes first straight and then actually concave (Fig. 78). Secondly, just posterior to the dorsal region of the gill plate there may often be noted a slight swelling, the outward indication of the internal growth of the

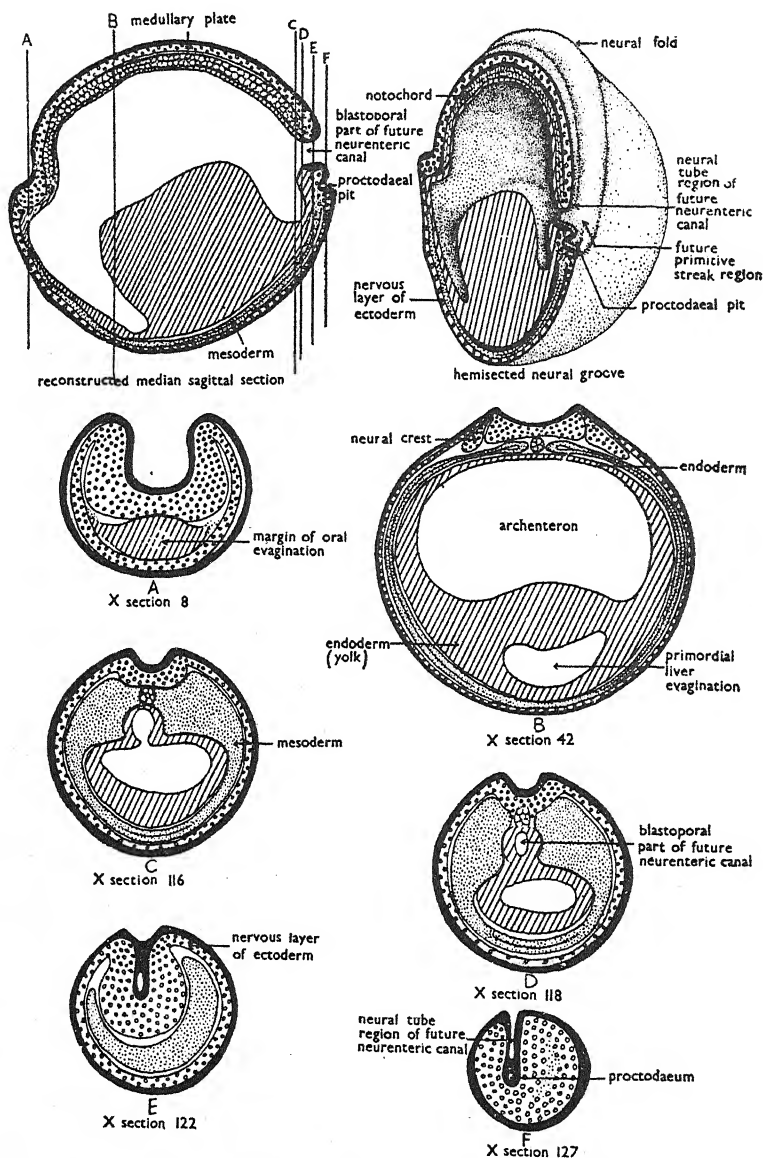


Fig. 80. — A median sagittal section reconstructed from serial cross sections, and a stereogram of a hemisected total neural groove stage. A, B, C, D, E, F. Selected cross sections as shown by serial numbers, at levels indicated by vertical lines on the sagittal section. The neural folds have not yet closed posteriorly to form the neurenteric canal and the primitive streak.

THE NEURAL TUBE AND RELATED PARTS 155

pronephros or embryonic *head kidney* (see below). Also along the dorso-lateral region posterior to the gill arches and just above the level of the *pronephros*, > shaped markings arise giving external evidence of the *myotomes*. Lastly the embryo by this time is partially covered by cilia whose motion causes it to rotate slowly within its membranes.

Under average outdoor conditions the stage thus described is generally reached at about the end of the second day after fertilization. Let us now turn to a consideration of the internal processes which have been going on during the same period.

INTERNAL CHANGES: THE NERVOUS SYSTEM

THE NEURAL TUBE AND RELATED PARTS

The Neural Tube. — This structure, as its name suggests, possesses an internal, laterally compressed canal termed the *neurocoel* or *neural canal*. From the manner of its formation, the lining of this canal is obviously the former outer ectodermal layer of the medullary plate, while the present outer wall of the tube was previously the inner or nervous layer of that plate. Thus the floor of the tube is relatively thin, since it occupies the position of the former medullary groove where the inner or nervous layer was least developed. The lateral walls, on the contrary, are thick because they are constituted of the well developed nervous layer on either side of the groove. The roof is evidently formed as the edges of the two folds meet one another and fuse, and, like the floor, it is thin as compared with the sides. As will appear below, this is due to the fact that not all of the nervous layer along the line of fusion becomes involved in that process. Finally it should be added that as the tube is thus made complete, the meeting of the folds likewise makes continuous the ectodermal wall above it.

The Neural Crests. — As just noted, not all of the nervous layer of the medullary plate is used up in the formation of this tube. The lateral edges of the plate, i.e., the neural ridges proper, although carried up to the region of dorsal fusion are not included in the walls of the tube. Instead, these ridges of nervous tissue are partially constricted off from the main part of the nervous layer. Each of the two ridges is thus semi-independent, and occupies a position well up in the angle between the sides of the tube and the ectoderm of the body wall (Fig. 80, *nc*). These are the *neural crests*, which presently become cut up into successive segments. In the head and branchial region the crests are quite prominent, but more posteriorly they are obscure and difficult to detect.

In general they are concerned with the development of the cranial and spinal ganglia, although those in the head and branchial regions have been found also to furnish material for some of the visceral arches. Their respective fates will be discussed in more detail in connection with the development of the nervous system.

THE BRAIN REGION AND SENSE ORGANS

The Brain Region.— In the anterior region the complete closure of the neural tube is somewhat delayed because of the greater breadth of the medullary plate at this point. Indeed, the process here might be still slower were it not that the growing together of the lateral edges is accompanied by the backgrowth of the transverse ridge. At the place where this ridge and the lateral folds are about to fuse there exists for a brief time a small opening; it is the *neuropore*, and is homologous with the similar structure in *Amphioxus*.

At the time the medullary plate first appeared, the embryo was still virtually in the form of a sphere, and the plate followed its curvature. As the neural tube begins to form, however, the embryo, as already noted, starts to lengthen out, the line of the back becoming straight, and then slightly concave. During this process, nevertheless, the original curvature in the foremost portion of both the neural tube and the notochord not only persists but even increases. It thus happens that these parts are bent downward so that the anterior and somewhat expanded extremity of the tube has the aspect of the bulbous closed end of a chemical retort. This bending is termed the *cranial flexure*. Hence it comes about that the roof of the tube in this region is actually anterior, and in the midst of this anterior wall is the recently closed neuropore. This point is marked by a slight invagination, both externally and in the brain wall, and by a small thickening in the nervous layer of ectoderm (Fig. 81).

Elementary Divisions of the Brain.— The constrictions which divide the brains of most vertebrate embryos into fore-brain, mid-brain, and hind-brain have not become evident in a 2.5 mm. Frog larva. These divisions of the brain may be roughly defined at this time, however, by reference to the following landmarks: Just opposite the curved anterior region of the notochord, the posterior wall of the brain, as suggested above, also curves, and the most anterior point on this curve may be designated as the *tuberculum posterius*. Directly across from this on the anterior brain wall is the invagination already noted as marking the closed neuropore, and immediately dorsal to this is a distinct in-

ward bulge formed by a mass of cells termed the *dorsal thickening* (Fig. 81). Using these points as places of reference the brain may now be divided into its three fundamental regions:

I. The *fore-brain* or *prosencephalon* extends from the anterior ex-

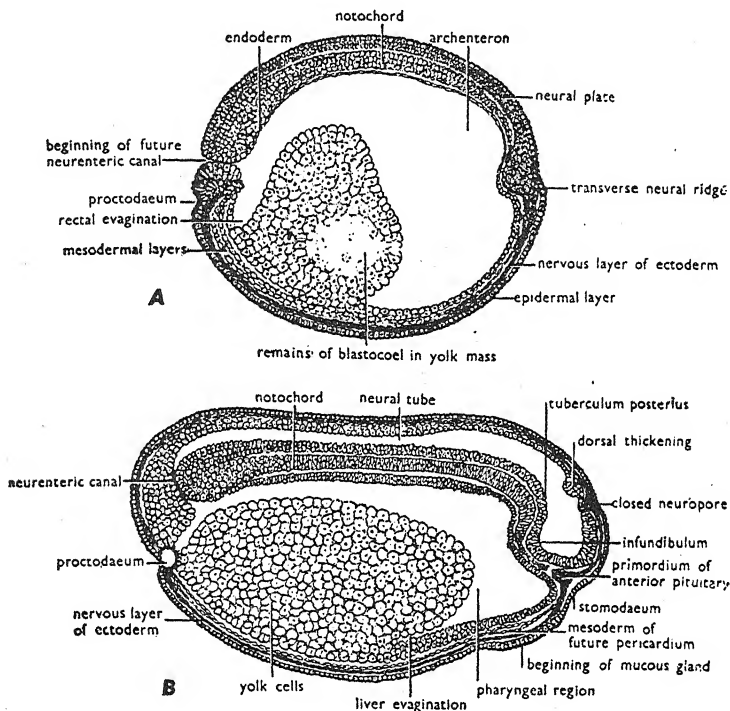


Fig. 81. — A. Sagittal section of neural groove stage. The remains of the blastocoel is not often seen so late as this. In this case the region between the beginning neurenteric canal and the proctodaeum (primitive streak) has been occluded by the fusion of the sides of the blastopore. B. Sagittal section of neural tube stage. The proctodaeum does not usually have so large a cavity connected with it, but did in this case. The rectal evagination which meets the proctodaeum is unlabeled.

tremity of the tube, i.e., the lowest part of the bent region, to a plane joining the tuberculum posterius with a point between the dorsal thickening and the closed neuropore.

II. The *mid-brain* or *mesencephalon* extends from the posterior boundary of the prosencephalon to another plane which joins the tuberculum posterius with a point slightly back of the dorsal thickening.

III. The *hind-brain* or *rhombencephalon* extends from the posterior boundary of the mesencephalon insensibly into the spinal cord.

It is thus evident, as indicated above, that the fore-brain is chiefly below and in front of the end of the notochord, the mid-brain is antero-dorsal to the end of the notochord, while the hind-brain lies entirely over the notochord.

Within the divisions of the brain thus defined there is very little differentiation of any sort as yet. In the most ventral portion of the forebrain, however, there does appear at about the end of the time we are considering, a slight rather broad and vaguely delimited posterior outpushing. It is the rudiment of the *infundibulum*, which will become the *posterior part* of the *hypophysis* or *pituitary body*. The *anterior part* of this important endocrine gland also appears at this time, and it is therefore convenient to describe it here, though unlike the posterior infundibular part it is not a brain derivative at all. At this stage it is more clearly defined than the infundibulum, and arises as a tongue of ectodermal cells of the nervous layer extending dorso-posteriorly from the dorsal margin of the stomodaeal invagination. It lies therefore just beneath the fore-brain, and is growing backward in such a way as eventually to meet the infundibulum (Fig. 81, B).

With the mention of these structures it becomes necessary to digress for a moment in order to make clear the way in which we shall use the terms applied to them and their parts. This is because the definitions of these terms have been considerably confused by various writers, especially as they have been employed in connection with some of the lower animals. Strictly speaking the organ referred to as the hypophysis or pituitary in human and other mammalian anatomy includes two main parts from the point of view of origin. One is derived from an ingrowth from the stomodaeum, and includes the *pars distalis* (anterior lobe proper), *pars intermedia* and *pars tuberalis*. The other main part is called the *pars nervosa*, which is derived from the larger portion of the infundibulum, the smaller remainder forming the stalk of the hypophysis. The *pars nervosa* is also frequently referred to as the posterior lobe (Gray's *Anatomy*, 24th edition). Even here, however, there is confusion since the "posterior lobe" according to some authors (Maximow and Bloom, 5th edition) seems to include not only the *pars nervosa*, indubitably of infundibular origin, but also the *pars intermedia* which is indubitably from the stomodaeum (Hegre, '46). As a matter of simplification, and for the purposes of this text, the writer will term all parts of the hypophysis derived from the stomodaeum simply the *anterior part*, and all parts derived from the infundibulum, i.e., the *pars nervosa*, the *posterior part*. Finally it should be understood that in the Amphibia

the position of the anterior part as here defined is really posterior to the pars nervosa or posterior part. The parts are nevertheless designated in this way because in adult avian, human and other mammalian anatomy the anatomically and embryologically homologous parts do actually occur in the anterior and posterior positions.

The Sense Organs.—Before the anterior or brain region of the medullary plate has closed, there appears on either side a patch of pigmented cells (Fig. 82). As a result of the closing process, these

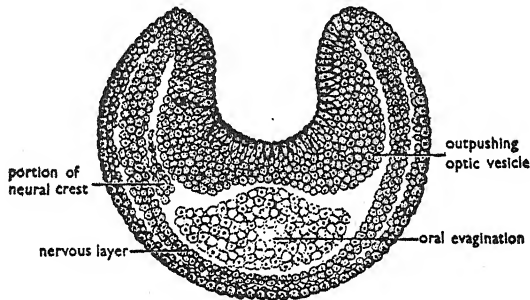


Fig. 82.—Cross section of a 2 mm. Frog embryo through the anterior end of the neural groove, showing optic vesicles starting to push out. Note the pigment spot on the inner side of each vesicle. The epidermal and nervous layers are thicker because they are cut tangentially due to the curve of the embryo in this region.

patches presently come to occupy positions on opposite sides of the interior of the fore-brain. The area of the brain wall including and immediately surrounding each patch now begins to push out or evaginate toward the external ectoderm of the head (Fig. 85, *A*). These evaginations are the *optic vesicles*. Presently each vesicle reaches the ectoderm in the dorso-lateral region of the sense plate, and by its pressure here soon causes a slight external protuberance noted above. Meanwhile the regions of the vesicles nearest the brain begin to become slightly constricted to form the *optic stalks* (Fig. 85, *A*).

The sensory portions of the ears, unlike the above parts of the eyes, do not develop from any region of the brain itself. Instead they arise from the dorso-lateral walls of the head. The rudiment of each appears during this period as a thickened patch of the nervous layer of ectoderm opposite the hind-brain. These thickenings in part constitute the *auditory placodes* (see below under ear).

At about the same time in another region of the head two other thick-

enings of the nervous ectoderm develop. In this case each is within the area of the sense plate a short space beneath, and, median to, the corresponding optic protuberance. These are the beginnings of the olfactory organs, and are termed the *olfactory placodes* (Fig. 83). Though later each is indicated externally by a pit, these markings are usually not in evidence at this stage (see below). Figure 83, however, is of a slightly later stage (3.5 mm.), which accounts for their appearance in that case.

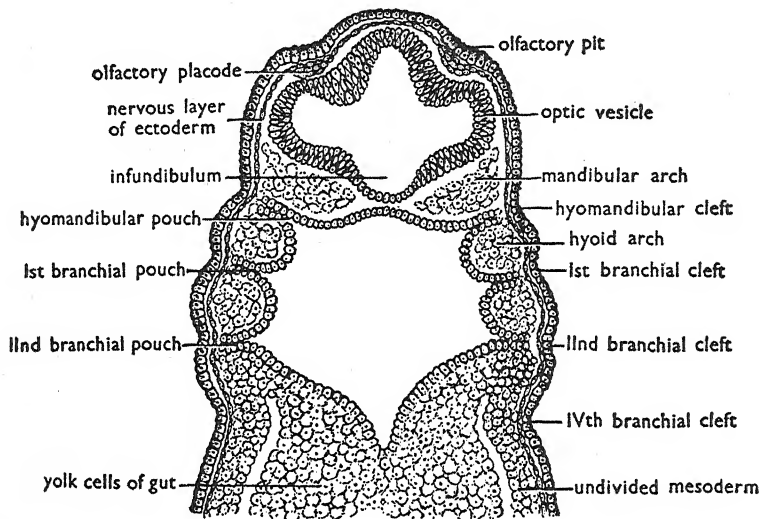


Fig. 83. — Frontal section of a 3.5 mm. Frog embryo through the olfactory pits, optic vesicles, and visceral clefts and pouches.

Experimental Results. — In connection with the discussion of gastrulation a good deal was said about the principle of induction, and it was indicated that further illustrations of it would be noted as occasion arose. Three excellent examples are afforded with respect to the origin of the oral mucous glands, the nasal capsules and the optic vesicles.

In the Urodele, *Amblystoma*,² it happens that in place of the mucous glands there occur leglike projections called balancers on which the ani-

² The writer is aware that the correct generic name for this animal is *Ambystoma* rather than *Amblystoma*. However, the latter has become so firmly fixed in the literature, particularly the embryological literature, that it seems best to use it in this text. This is made even more advisable in view of the fact that the latter spelling is the one used in all the articles cited.

mal rests. Schotte and Edds ('40) found that Frog ectoderm from regions which would not normally produce mucous glands, would do so when transplanted to the head of *Amblystoma* at the site of, and in place of, the latter animal's balancer producing ectoderm. This shows two things. It indicates first that the formation of either mucous glands or balancers is apparently due to the inductive action of the underlying mesoderm. Secondly, it shows that though the Frog ectoderm is thus acted upon by the *Amblystoma* inductor, it can, nevertheless, only form the kind of organ for which it has competence, namely, mucous gland, not balancer.

In the case of the nasal placodes Zwilling ('40) has shown among other things that apparently they may be induced in the nervous ectoderm by the roof of the underlying archenteron. Also the olfactory pit can be induced in the epidermal ectoderm by the layer of nervous olfactory ectoderm underlying it.

Finally in the case of the optic vesicles Adelmann ('30, '37) and others, by the usual transplantation experiments, have demonstrated two points. First, the inherent capacity (competence) of the head ectoderm to form these vesicles at all is considerably reinforced by the inductive action of the underlying prechordal plate (potential notochord). Secondly, this inductive action causes two vesicles to form where there would otherwise be only one (cyclopia).

THE NEURENTERIC CANAL

While the above developments have been taking place in connection with the anterior end of the nervous system there has also been a change posteriorly. It was noted in describing the externals that as the neural folds close in this region, they roof over the dorsal part of the blastopore. As stated, however, this portion of the blastopore, though no longer communicating with the outside, still remains open. It thus constitutes a temporary connection between the enteron and the neurocoel. As in *Amphioxus*, this connection is termed the *neurenteric canal* (Fig. 81). It should be noted in this case that the canal is seldom if ever demonstrable as an actual open tube, and its existence has therefore been denied by some. Usually in fact it appears merely as a line of pigment. In good specimens which the writer has examined, however, the clean cut character of the cells bordering the path of the "canal" in all probability indicates a definite line of cleavage. Indeed it seems clear that what amounts to a "probe patency" certainly exists, were it possible to use a probe on so small a structure.

INTERNAL CHANGES: THE ENTERON

THE FORE-GUT

The anterior region of the archenteron is enlarged and lies in front of the mass of yolk cells which form the floor of the middle region. This anterior portion is therefore termed the *fore-gut*, and a little later will be differentiated into the pharynx, esophagus, stomach, and liver. These parts are as yet scarcely distinguishable. Nevertheless, during the period under discussion, the fore-gut as a whole gives rise to certain rudiments as follows:

The Pharyngeal Region. — In the antero-ventral region beneath the fore-brain there is an outpocketing in the direction of the invaginated ectoderm, though the two walls are not yet in contact. It is called the *oral evagination* and may be considered as the extreme anterior end of the *pharynx* (Figs. 81; 85, B). Immediately posterior to this in the region of the fore-gut which is destined to become the pharynx proper there have already been noted the external rudiments of certain of the visceral clefts; i.e., the *hyomandibular*, and the first, second, and fourth branchials. Considering now the internal development of this region at a corresponding stage, the following condition is to be observed. Opposite the invaginating ectoderm which marks externally the rudiments of the above mentioned clefts the endoderm of the pharynx is beginning to push outward upon either side to form the corresponding pairs of *hyomandibular*, and *first* and *second branchial* or *gill pouches*. It should further be added that although these vertically elongated pharyngeal evaginations are called pouches, they do not actually appear as such. This is because the anterior and posterior walls of each outpushing are at this time fused together, so that no pouch cavity really exists. Thus it may be noted that each pouch resembles rather a two layered sheet of endoderm, extending from the fore-gut toward the ectoderm (Figs. 83, 102).

The Liver. — In the extreme ventro-posterior part of the general pharyngeal region there is evident a slight posteriorly directed pocket beneath the anterior end of the yolk mass. This represents the rudiment of the *liver* (Fig. 81, B).

THE MID-GUT

The portion of the enteron following the fore-gut lies, as noted, above the main mass of the yolk cells which thus form its floor. Its lumen is

relatively small with a thin roof, and sides which thicken ventrally. It is the *mid-gut*, and is destined later to develop into the intestine.

A peculiar and transitory structure developed in connection with this region is the *hypochordal rod*. It arises at about 2.5 mm. as a longitudinal string of cells constricted off from the dorsal wall of the mid-gut, between it and the notochord. Appearing first slightly posterior to the pharyngeal region it later extends even into the tail. It soon becomes separated from the gut by the development of the dorsal aorta, and shortly after hatching it disappears entirely.

THE HIND-GUT

Posterior to the mid-gut just in front of the neurenteric canal the enteron enlarges slightly. This region is termed the *hind-gut*, and is destined to form the rectum.

THE MESODERM AND RELATED STRUCTURES

Shortly following gastrulation, the condition of the mesoderm is as follows: Ventrally and laterally it exists as a continuous sheet extending up to the notochord on either side. In the head and most of the pharyngeal region it is represented only by scattered cells, while posteriorly it reaches to the blastoporal region, which continues to bud it off. During the period we are now discussing the mesoderm thus indicated begins to give rise to various structures in the following manner:

THE VISCERAL ARCHES

It will be recalled that in the pharyngeal region at this time the hyomandibular and the first two pairs of branchial or gill pouches are developing as solid vertically elongated evaginations of endoderm. As these evaginations push out to the ectoderm, it is obvious that the mesoderm in the way of each will be thrust to either side. In this manner such mesoderm becomes more or less concentrated in the regions of the future visceral arches which are to alternate with the pouches. Indeed, it may at this time be said to represent their rudiments, whose external appearance has already been described, as having the form of raised areas between the incipient clefts. Thus in front of the first or hyomandibular pouch is the mesodermal rudiment of the *mandibular arch* (apparent externally as the lower portion of the sense plate upon either side of the stomodæum), while between the hyomandibular and first branchial pouch is the rudiment of the *hyoid arch*. The *first bran-*

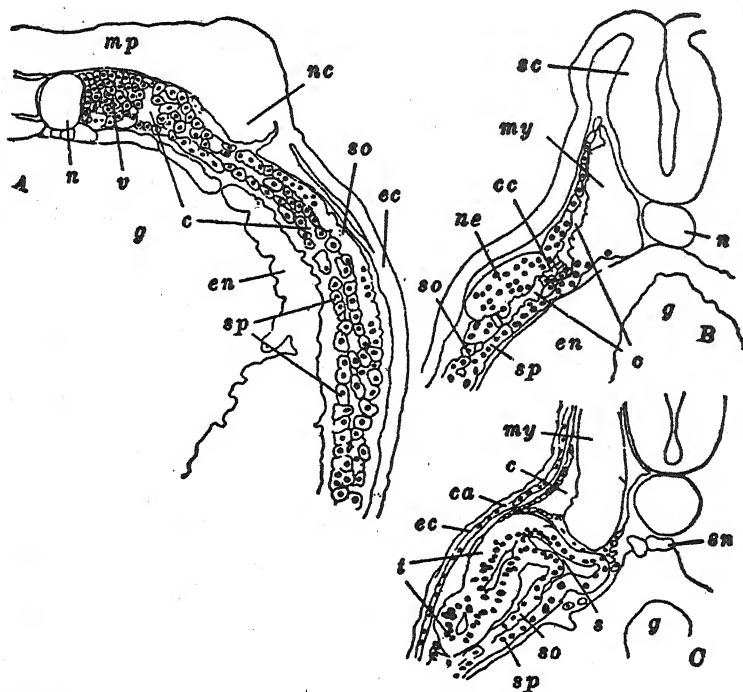


Fig. 84. — Sections through Frog embryos (*R. sylvatica*) illustrating the formation of the pronephros. From Kellicott (*Chordate Development*). After Field. *A*. Through the anterior body region of an embryo at the commencement of its elongation. *B*. Through the anterior end of the pronephric rudiment of an embryo in which the neural folds are just closed together. *C*. Through the second nephrostome of an embryo of about 3.5 mm.

c. Coelom. *ca*. Rudiment of pronephric capsule. *cc*. Communicating canal. *ec*. Ectoderm. *en*. Endoderm. *g*. Gut cavity. *mp*. Medullary plate. *my*. Myotome. *n*. Notochord. *nc*. Rudiment of neural crest. *ne*. Nephrotome. *s*. Pronephric nephrostome. *sc*. Spinal cord. *sn*. Subnotochordal rod (hypochorda). *so*. Somatic layer of mesoderm (in *A* the reference line points to the rudiment of the pronephros). *sp*. Splanchnic layer of mesoderm. *t*. Pronephric tubule. *v*. Vertebral plate of mesoderm.

chial arch then follows the first branchial pouch, and the *second branchial arch* follows the second branchial pouch. Since, however, the third branchial pouch is scarcely formed as yet, the mesodermal element of the second branchial arch is not at this time very clearly distinguishable from the tissue posterior to it.

THE SEGMENTAL PLATES AND THE LATERAL PLATES

Along either side of the notochord posterior to the pharyngeal region, the mesodermal sheet thickens into a relatively narrow band which

is termed the *segmental* or *vertebral plate*. The remainder of each sheet below this region is then called a *lateral plate*. Ventrally the two lateral plates are continuous with one another (Fig. 85, *D*).

Formation of the Coelom. — In its dorsal region each lateral plate now begins to become split into two sheets. The outer sheet next to the

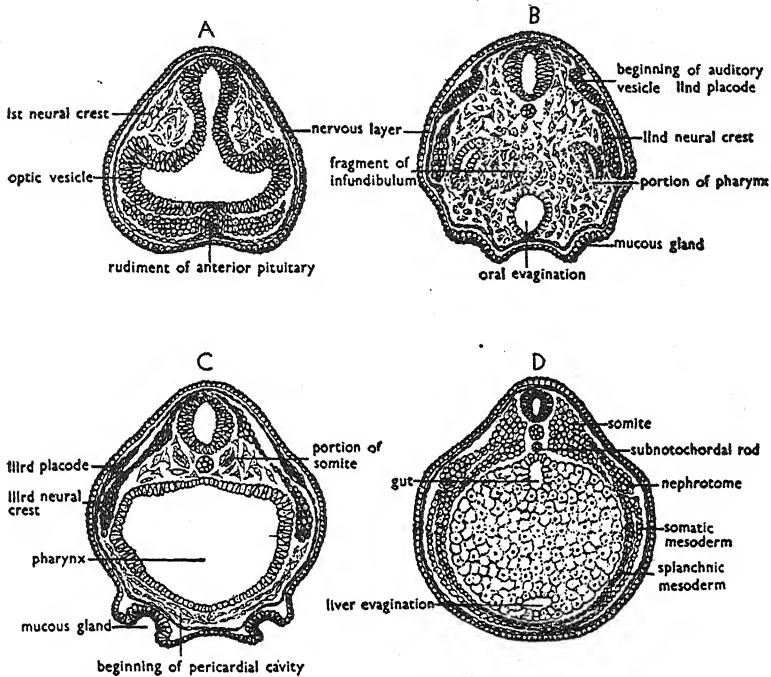


Fig. 85. — Four selected cross sections from a series of one 2 mm. (neural-tube stage) Frog embryo. *A*. Through the optic vesicles and rudiment of anterior pituitary. *B*. Through auditory vesicles and oral evagination. *C*. Through pharynx in region of 11 neural placodes and crests, and the future heart. *D*. Through anterior part of mid-body region, showing liver evagination and nephrotomes.

ectoderm is the *somatic mesoderm* (somatopleure), while the inner sheet next to the enteron is the *splanchnic mesoderm* (splanchnopleure) (Fig. 85, *D*). Between them a space presently becomes evident which is the rudiment of the *coelom*. Upon either side, this coelom then gradually extends downward through its respective lateral plate. During the period we are describing, however, these two extensions do not reach quite far enough to meet one another beneath the gut. Thus in this region the coelomic cavity in each plate is temporarily separated from the one on the opposite side. Besides this downgrowth of these cavities

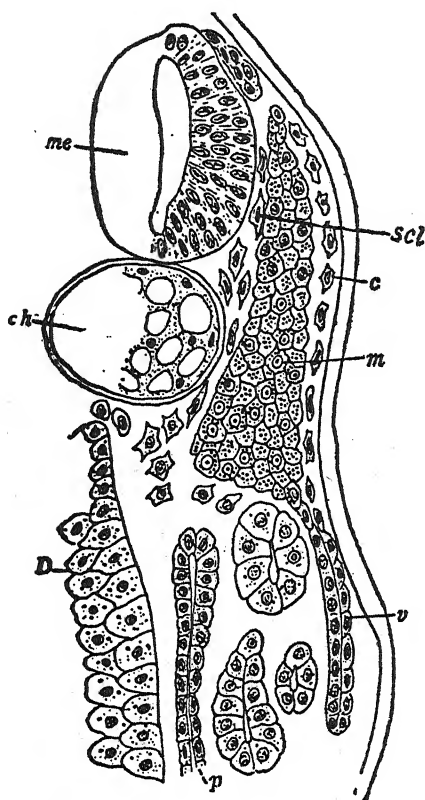


Fig. 86. — Transverse section through the sixth mesodermal somite of a 5 mm. larva of *R. temporaria*, illustrating the arrangement of the mesoderm. From Kellicott (*Chordate Development*). From Maurer (*Hertwig's Handbuch*, etc.).

c. Cutis plate. ch. Notochord. D. Gut wall. m. Myotome (muscle plate). me. Nerve cord. p. Lateral plate. scl. Sclerotomal cells. v. Ventral process of myotome and cutis plate.

the somite is called the *myotome*. The differentiation between these parts is often indistinct at this time (Fig. 85, D), but is usually clearer at a later stage (Fig. 86).

THE NEPHROTOME

Along the dorsal border of each lateral plate, just at the line of separation between lateral plate and segmental plate, is a narrow strip of

there is also an upgrowth into the mesoderm of the segmental plates (Fig. 84). Here the slight spaces which last but a brief time are termed the *myocoels*.

The Somites. — Meanwhile the segmental plates are also undergoing other changes. Just back of the pharynx each plate is being divided transversally into sections termed *somites*. During the period under consideration, about four pairs of these somites are thus formed, development proceeding posteriorly. Shortly after its formation each somite loses its connection with the lateral plate, and exists as a separate mass of cells. Within each somite so isolated the myocoel may persist for a brief time, not at the center of the mass, but just beneath the outer surface. Because of its previously supposed subsequent history (see below) the thin layer of cells forming this outer surface is termed the *cutis plate* or *dermatome*. For the same reason the remaining inner part of

somatic mesoderm which is destined to form both the larval and adult excretory systems. This strip is termed the *nephrotome*, and becomes evident as such very early (Fig. 84, *B*; Fig. 85, *D*). Indeed, even before separation of the above plates this region begins to proliferate cells between itself and the ectoderm. In this way the nephrotome becomes a thick band of tissue attached along its inner border to the dorsal edge of the lateral plate, whose side it overhangs slightly, like the eave of a roof. At the very first, as segmentation appears in the vertebral plate, it also extends slightly into the nephrotomal band. Thus the single nephrotome tends to become divided into a series of nephrotomes. This division, however, is very transitory in the Frog and disappears without further significance. As the coelomic split begins to appear in the lateral and segmental plates, spaces also start to form in the nephrotome from about the second to the fourth somites (Fig. 84, *C*). This marks the beginning of the *pronephros*, the evidence of whose presence has already been noted in the description of the exterior.

THE BEGINNING OF THE PERICARDIAL CAVITY AND THE HEART

In the region of the pharynx it has been indicated that laterally the rather loosely arranged mesoderm is involved in the formation of the gill arches. In the floor of this region, however, uniting the ventral ends of these arches, there is a sheet of mesoderm coextensive posteriorly with the fused lateral plates. It will be recalled that at this period the down-pushing coelomic spaces in these plates have not reached to the ventral side of the animal. Anteriorly, however, in the ventral portion of the mesodermal sheet which lies beneath the pharyngeal floor, there may now appear a slightly indicated pair of independently developing spaces (Fig. 85, *C*). Each space lies within the sheet upon either side of the mid-line, the two spaces being separated from one another by a narrow median strip of the mesoderm which remains undivided (Fig. 85, *C*). These spaces are the rudiments of the *pericardial cavity*, whose walls are termed the *pericardium*. The outer or parietal wall is indicated at present by the lower of the two mesodermal sheets. It is continuous, both now and in the completed organ, with the inner or visceral wall which arises from a portion of the upper sheet, and which eventually forms a closely adherent covering for the heart muscle. (See Fig. 85, *C* and *D*; cf. also Fig. 107.)

Just above the median strip, between it and the endodermal floor of the pharynx, there may also appear at this time a few scattered cells.

These cells have been regarded as having originated like the dorsal mesoderm of the lateral plates, i.e., by a splitting off from the endoderm which in this case lies above them. It now appears, however, that they are derived entirely from mesoderm which, in *Amblystoma* at least, as shown by staining experiments of Wilens, '55, has migrated from between the ear anlage and the hind-brain. The scattered cells are destined to form the endothelial lining of the heart, or *endocardium*, while the remainder of this mesoderm forms other heart and pericardial tissue to be described in the following chapter (Fig. 85, C). Though all parts of the heart are thus apparently mesodermal in origin, there is evidence that the overlying endoderm does have an organizing effect on their development (Bacon, '45).

Before leaving the development of the heart at this early stage, it is of interest to note what happens when these heart-forming elements are manipulated in various ways as was done by Copenhaver ('26) on *Amblystoma*. Thus if a moderate amount of the median region is removed, the lateral parts will grow down and replace it so that a single complete heart develops. If, however, a piece of foreign mesoderm is substituted for the removed part, the lateral parts will form two separate hearts with mirror image symmetry. Removal of an anterior or posterior half does not prevent the formation of a complete heart if it is done early enough, but the anterior and posterior parts are apparently irreversibly determined considerably sooner than are the lateral parts. Not only, however, is it true that parts may form whole hearts, but two wholes if properly united may form single hearts. Thus if a second layer of heart-forming mesoderm from one embryo is superimposed by transplantation upon the heart-forming mesoderm in another embryo, the two layers will fuse and a single normal heart develops. On the other hand, as might be anticipated from the previous statement about anterior posterior determination, this only happens at the stage in question if the second layer is normally orientated. If the latter is reversed with respect to its antero-posterior axis, fusion is imperfect. Also, since heart pulsation is initiated at what is at first the posterior end of the organ, in this latter case disharmonic pulsation results.

T

HE FROG: LATER OR LARVAL DEVELOPMENT

IN the last chapter the development of the embryo was discussed up to the point where it had reached a length of about 2.5–3 mm., and acquired the rudiments of most of the chief systems and organs. We shall now continue the history of the animal from this point to the adult condition, having regard to both the external and internal changes. The former will be considered first, under the head of three rather obvious stages which will become apparent as the description proceeds.

EXTERNAL DEVELOPMENT

TWO AND ONE-HALF MILLIMETERS TO HATCHING

During the first week or two, depending on the temperature, elongation progresses to a considerable extent, largely as a consequence of the outgrowth of the tail region posterior to the blastopore. Concurrent with this process, the > shaped depressions marking the boundaries of the myotomes not only become evident throughout the body region, but appear also upon the sides of the tail. At the same time just back of the gill plates the pronephric swellings increase in size. In the head the outpushings due to the optic vesicles become somewhat more pronounced, but in a slightly different position from the one which they first occupied, i.e., less upon the front of the head and more upon the side. This last mentioned change is really due to the beginning of a forward growth of the region anterior to them, which continues gradually for some time, and results in the eventual location of the eyes some distance from the tip of the snout. Meanwhile the *stomodaeum* proper forms at the dorsal end of the elongated stomodaeal invagination, while upon each sense plate, slightly dorsal and to one side of the stomodaeum, appears a small depression, the *olfactory pit*. Each gill plate, on the other hand, now develops upon its surface another slight vertical groove lying between the rudiments of the second and fourth branchial clefts. This new indentation is the beginning of the *third branchial cleft*, so that the positions of all four branchial clefts are now indicated (Fig.

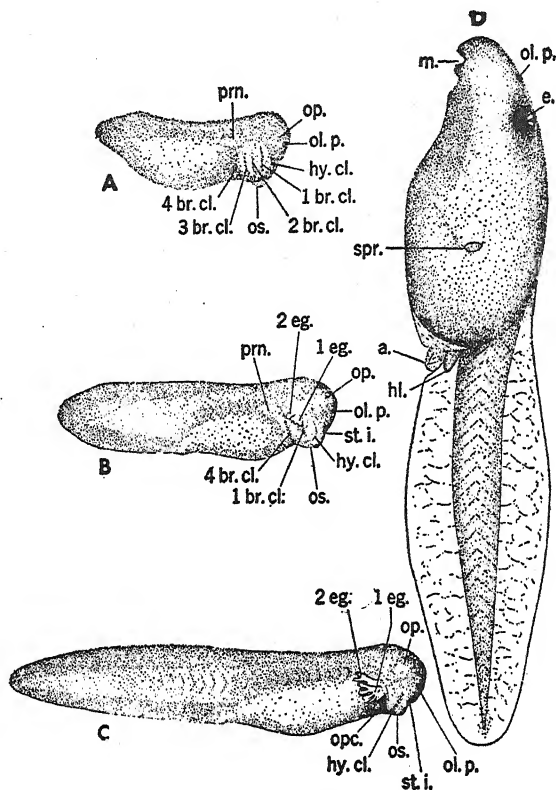


Fig. 87. — Drawings of preserved Frog embryos and larvae (*Rana pipiens*) from 4 mm. to 14.5 mm. in length. For the sake of keeping correct the relative size differences of the drawings in this figure it has been necessary to make them on a smaller scale than those in figure 78. *A.* Right side of a 4 mm. embryo. It will be noted that the tail has just begun to grow out, that the positions of all the visceral clefts are apparent, and that the olfactory pits are present. The oral "suckers," being now entirely ventral, are not actually visible from this point of view. The myotomes in this embryo and in *B* and *C* are very slightly indicated externally. *B.* Right side of a 6 mm. embryo. The external gills of the first and second branchial arches have begun to develop, concealing the second and third branchial clefts. The stomodaeal invagination is deepening, and is slightly visible from the side. *C.* Right side of a 9 mm. embryo. The external gills have grown considerably, and developed several lobes. From the posterior border of the lower portion of the hyoid arch, the operculum is just starting to develop, and thus covers slightly the region of the first branchial cleft. The stomodaeal invagination, scarcely visible from the side, has almost given rise to the mouth. *D.* Left side of a 14.5 mm. larva. The external gills have been covered by the operculum, and the gill chamber opens to the outside only through the spiracle. The eye is formed, the mouth is opened into the pharynx and its lips are covered with rasps. The hind limb buds have appeared, and the tail has developed a finely veined membranous edge or fin.

a. Anus. *1 br.cl.* *2 br.cl.* *3 br.cl.* *4 br.cl.* Rudiments of the first, second, third, and fourth branchial clefts. The corresponding arches and their positions are indicated in the text. *e.* Eye. *1 eg.* *2 eg.* First and second external gills. *hl.* Hind limb buds. *hy.cl.* Rudiment of hyomandibular cleft. *m.* Mouth. *ol.p.* Olfactory pit. *op.* External indication of optic vesicle. *opc.* Edge of operculum. *os.* Oral "sucker." *prn.* External indication of pronephros. *spr.* Spiracle. *st.i.* Stomodaeal invagination.

87, A). Lastly, a short time before hatching there appears upon the upper part of the first and second branchial arches of each side a small lobed outgrowth; the rudiments of two pairs of *external gills* (Fig. 87, B).

The embryo (6-7 mm.), which is now ready to hatch, presently wriggles its way out of the surrounding jelly. From this time on it may be referred to as the *larva* or *tadpole*.

FROM HATCHING TO METAMORPHOSIS

Early Larval Life. — For a few days after hatching, the young tadpole, which is a dark brownish color, lies on its side or remains attached to some convenient object by its V-shaped mucous gland. During the first part of this period the mouth is incompletely formed, and the animal is still dependent on the yolk for its nourishment. Meanwhile the two pairs of external gills develop rapidly, the original lobes of each gill putting forth several longer minor lobes or filaments (Fig. 87, C). There furthermore arises upon each third branchial arch a rudimentary third gill. This gill, however, never develops far, and is overlapped and concealed by those anterior to it. Aside from these features it will also be noted that the body and particularly the tail have increased in length, while the optic protuberances are still further back, as a result of the continued outgrowth of the snout. Upon the center of each of these protuberances, moreover, there frequently appears at this time a slight depression marking the external beginnings of the actual eyes which are soon clearly visible.

In another week or somewhat less (9-10 mm.), certain further changes occur as follows. The mouth is opened and appears as a small round orifice armed with a pair of horny jaws and with lips covered by horny rasping papillae. At the same time the above mentioned mucous gland begins to atrophy, and the larva giving up its fixed existence swims actively about in search of food. This consists of either animal or vegetable debris which it can scrape loose with its horny jaws and lips; in captivity it will feed readily on any sort of cereal. In connection with this change of nourishment, the digestive organs are rapidly developed so as to give the body a full rounded appearance. This is particularly due to the great increase in the length of the intestine which can be seen through the ventral body wall looking like a coiled spring.

As the above alterations occur in connection with the alimentary tract, certain changes also take place in the respiratory system, of which the following may be regarded as exterior. Posterior to the first and second branchial arches the incipient second and third branchial clefts be-

come opened into the pharynx by way of the corresponding pouches as actual clefts or gill slits. The first and fourth branchial depressions then presently become true clefts in a similar manner. Concurrent with these events there is also developing from the posterior border of each hyoid arch a fold of integument called the *operculum*. These opercula then grow backward on each side, covering the gills as they progress. They also grow toward one another ventrally until they meet and fuse. Thus a closed *branchial* or *gill chamber* is formed which opens externally on the left side only, through a short funnel between the body wall and operculum, known as the *spiracle* (Fig. 87, D). It should finally be noted in this connection that as the closure of the branchial chamber is completed, the external gills start to atrophy and are replaced by internal gills upon the edges of the gill slits. These new organs will be more fully described in the discussion of internal changes.

Later Larval Life.—After the attainment of the above condition during the first two or three weeks of larval life, development proceeds somewhat more gradually to the time of metamorphosis. During this interval, which may last for two or three months or sometimes over the following winter, the larva increases considerably in size.¹ It also loses its brownish color and becomes more or less green dorsally, and white ventrally. Perhaps the most striking external feature, however, is the growth of the legs which begins at about the end of the first month. The fore legs develop first, but are not visible because they are covered by the operculum. The hind legs are easily seen as they arise at the base of the tail, and by the end of the second month they begin to show joints.

Experimental Results.—In connection with leg development a considerable amount of experimentation has been done to discover when the antero-posterior and dorso-ventral axes are determined, and what factors may be involved in the process. These experiments have been made on *Amblystoma* rather than the Frog, but it seems likely that results would be quite similar in the latter animal. The procedure consisted in reorientating the forelimb rudiment either in the normal (orthotopic) location or in some abnormal (heterotopic) location. Thus Harrison ('21) found that if in an embryo with a small tail bud (stage 29) this limb rudiment were implanted dorsal side down in its normal place it would develop a limb with the dorsal side up, but with the antero-posterior axis reversed. Eventually of course a stage would

¹ The larval condition is said to be prolonged by a cool season or a scarcity of food. Also the larva of certain species, i.e., the Bull Frog, *Rana catesbiana*, normally passes through the winter before metamorphosis.

be reached where the dorso-ventral axis also could no longer adjust itself following inversion, but that obviously occurs at a later period.

Other workers have confirmed and amplified these conclusions. Thus Swett ('37, '39, '41) showed that subsequent reversal of the dorso-ventral axis of a previously inverted limb is apparently due to factors in the flank region, since inverted rudiments implanted in the region of the myotomes remain inverted. Also it appears that the effect of these flank factors may be blocked if tissue dorsal to the limb rudiment is included in the inverted implant.

It should be realized of course that all these cases are again simply illustrations of special instances of the effect of one part upon another, i.e., induction.

METAMORPHOSIS

Usually under normal conditions the tadpoles of most species begin to frequent the surface of the water during the third month. Here they expel bubbles and gulp in air to supply the developing lungs. This is one of the signs that metamorphosis is near at hand, and at about the end of this month the final changes to the form of the adult Frog generally occur with relative rapidity.

These changes are both internal and external. The former will be described more fully later. They involve, however, a complete development of the lungs accompanied by certain changes in the circulatory system. There is also an enlargement of the stomach and liver, and at the same time a great shortening of the intestine. This change is apparently correlated with the carnivorous habits assumed by the adult. Externally the alterations are no less fundamental, and perhaps even more striking. The larval skin is cast off, and with it the horny jaws. The frilled lips likewise disappear and the mouth instead of being round becomes very wide. The tongue enlarges, and the eyes grow more prominent. The fore legs become visible by being thrust through the operculum. The left appears first because it extends through the respiratory funnel on that side, while the right is forced to break through the opercular wall. At the same time, in company with the development of the lungs, the gills dry up and the gill slits opening into the opercular chamber are closed. The hind limbs, which have long been visible, increase greatly in length, and the tail is rapidly absorbed. Sexual differences both internal and external now become clearly evident. There are other minor changes, but those cited comprise the more prominent and important ones.

The changes just described have of course been known for a very long time. It is only within recent years, however, that some of the activating factors have been uncovered by numerous experimenters. By appropriate removal, transplantation, and injection operations it has been pretty thoroughly demonstrated that as in the case of so many other bodily functions the prime mover of metamorphosis, so to speak, is the pituitary gland. This small, though extremely important, endocrine gland starts to hypertrophy as the time of change approaches. It, or more specifically the anterior part of it, then secretes a hormone which in turn activates the thyroid. The latter responds by secreting the thyroxin which in this case brings about the various metamorphic changes characteristic of the particular tissue and the specific animal concerned, B. M. Allen ('32), Atwell ('35), Atwell and Holley ('36), Etkin ('36), Etkin and Huth ('39), Figge and Uhlenhuth ('33) and others.

In addition to this evidence as to the internal secretions involved in metamorphosis there have also been numerous experiments indicating how different tissues respond to the change in general body environment brought about by the endocrines. Thus Helff ('29, '30) has shown that tail muscle transplanted to the back atrophies at the time that the rest of the tail disappears, and the same has been demonstrated for the tail skin by Lindeman ('29). This might be anticipated, but it is more significant that back muscle and skin transplanted to the tail does not atrophy with the latter. Instead it simply moves up onto the back. An even more striking example of this is the case of eyes transplanted to the tails. In several successful operations the eye also moved up at metamorphosis, and appeared on the rear end of the Frog (Schwind, '33, Fig. 88).² These are situations with respect to tissues occurring within a single species. Another revealing result is obtained when Frog tail buds are transplanted to *Amblystoma* larvae. At metamorphosis, when the *Amblystoma* loses its tail fin, though not of course its tail, the well-developed Frog tail entirely disappears (as reported by Goldsmith at A. A. A. S. meeting '33).

Thus in all these cases it seems clear that the fundamental bodily condition brought about by the endocrine secretions is similar. What differs is the kind of tissue. Indeed different tissues in the same animal obviously must differ in this respect, else a general condition causing

² Though not stated, it is scarcely possible that these eyes were functional, even though pieces of brain were present in some cases. Hence these remarkable specimens were probably not blessed with both foresight and hindsight!

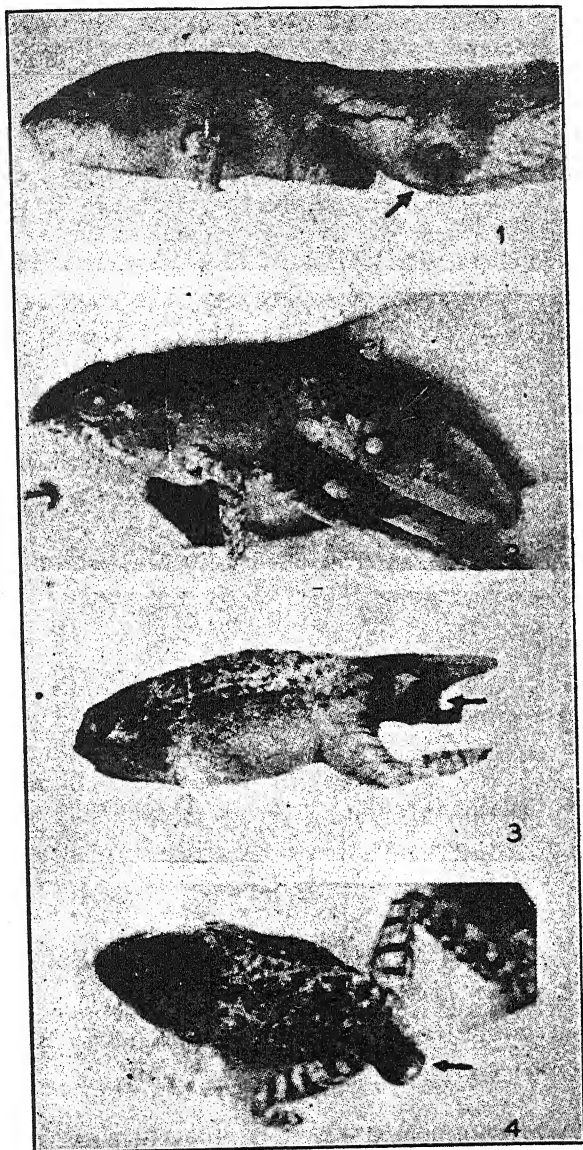


Fig. 88.—Photographs of stages in the metamorphosis of a Frog tadpole which had had an optic vesicle transplanted from another larva to the region of the tail at the tail-bud stage. Both tail and vesicle developed normally. Then when the tail was absorbed, the fully formed eye persisted, and was moved forward to the posterior end of the animal. Why was the eye not also absorbed? See text.

176 THE FROG: LATER OR LARVAL DEVELOPMENT

the atrophy of one would cause the atrophy of all. In that event not only would the tadpole tail disappear at metamorphosis, but the whole tadpole would vanish like the famous cat in Alice in Wonderland. Evidently likewise the difference in the behavior of similar structures, e.g., the tails in the Frog and in *Amblystoma*, is due to specific tissue differences in these structures.

These activities, it may be noted, are in some sense different from the inductive effects which have previously been cited as playing so fundamental a part in development. The difference, however, is probably not very significant. It must be assumed that in the case of endocrine activities the effects are, or may be, produced on tissues at some distance from the source of the inducing agent, in such instances called a hormone. In the cases of induction previously noted one must likewise assume the production of some chemical substance which produces its characteristic effects. Only in these latter instances the inducing agent "hormone" is only active upon tissues in contact with or very close to its source. There are further striking illustrations of the latter type to be found in connection with metamorphosis. One of these is the case of the histolysis of the opercular skin over the outpushing forelimb. This skin is partly broken by the pressure of the limbs. However, Helff ('26) has shown that the histolytic action which aids this breakthrough is produced by the atrophying gills in the immediate vicinity.

We have now finished our survey of the external changes in the embryonic development of the Frog. In the description of internal changes, it will be most convenient, in so brief a discussion, to complete entirely the history of one system before taking up the next. In the case of each, however, as many references as possible will be made to the stages noted in the account of the exterior. With this aid the student is urged to correlate as often as possible the condition reached by one group of organs with that reached by another, as well as with external changes. Only in this way is it possible to obtain a true conception of the growth of the animal as a whole.

INTERNAL DEVELOPMENT: THE NERVOUS SYSTEM

THE BRAIN

When last mentioned, this organ had been somewhat artificially divided into fore-, mid-, and hind-brain, and within the fore-brain the rudiment of the infundibulum was vaguely outlined. Further development in the three divisions now occurs as follows:

The Prosencephalon. — Somewhat previous to hatching, at about 4 mm., certain structures have developed which are characteristic of Vertebrate brains at early stages, and which are clearly evident in median sagittal sections as follows: To begin with the rudiment of the infundibulum already noted has become somewhat more pronounced. Proceeding anteriorly around the ventral side of the fore-brain, we encounter next a slight thickening, separated from another more anterior thickening by a narrow region where the wall is thin, giving the effect

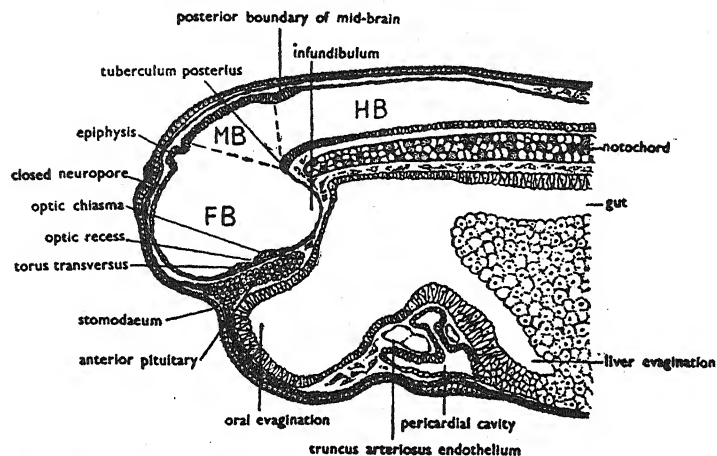


Fig. 89. — Median sagittal section of the anterior end of a 4 mm. Frog embryo. *FB*. Fore-brain. *MB*. Mid-brain. *HB*. Hind-brain.

of a depression. The posterior thickening next to the infundibulum is the rudiment of the *optic chiasma*, though of course no nerve fibers are present in it at this time. The thin region anterior to it is the *optic recess*, and the more anterior thickening is the *torus transversus*. Continuing up unto the anterior wall of the fore-brain, we see a distinct though narrow outpushing slightly dorsal to the end of the notochord. It is the epiphysis (Figs. 89, 90).

As regards other developments in this region of the brain we find that at about the time of hatching there grows out from the anterior end of the fore-brain a thin-walled vesicle, which represents the rudiment of the *cerebrum*. Presently its sides become thickened, and somewhat later (12 mm.), it is partially divided in two by a median longitudinal invagination of the anterior and the dorsal wall. The laterally compressed cavities of the resultant halves, or *cerebral hemispheres*, are then known as the *lateral ventricles*. Posteriorly they communicate with the main

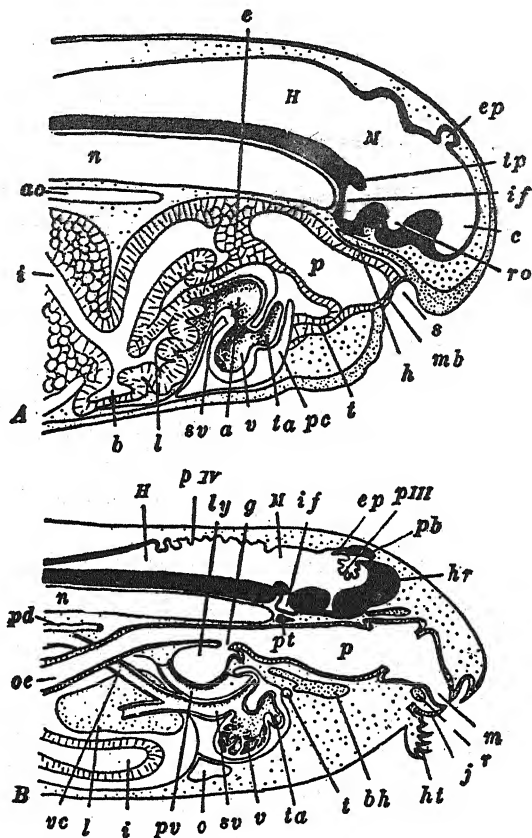


Fig. 90. — Diagrams of median sagittal sections of the anterior ends of Frog larvae. From Kellicott (*Chordate Development*) After Marshall (*Vertebrate Embryology*, courtesy of Putnam's Sons) *A*. Of a larva just before the opening of the mouth. *B*. Of a 12 mm. larva (at the appearance of the hind-brain buds).

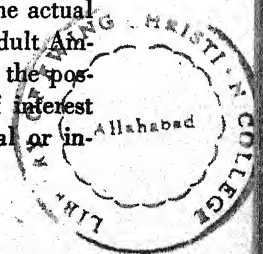
a Atrium. *ao*. Dorsal aorta. *b* Gall bladder. *bh*. Basihyal cartilage. *c*. Cavity of rudimentary cerebrum. *e*. Epithelial plug closing the oesophagus. *ep*. Epiphysis. *g*. Glottis. *h*. Hypophysis. *H*. Hind-brain. *hr*. Cerebral hemisphere. *ht*. Horny "teeth." *i*. Intestine. *if*. Infundibulum. *j*. Lower jaw. *l*. Liver. *ly*. Laryngeal chamber. *m*. Mouth. *M*. Mid-brain. *mb*. Oral membrane (oral septum). *n*. Notochord. *o*. Median portion of opercular cavity. *oe*. Esophagus. *p*. Pharynx. *pb*. Pineal body. *pc*. Pericardial cavity. *pd*. Pronephric (more posteriorly mesonephric) duct. *pt*. Pituitary body. *pv*. Pulmonary vein. *pIII*. Choroid plexus of third ventricle. *pIV*. Choroid plexus of fourth ventricle. *r*. Rostral cartilage. *ro*. Optic recess. *s*. Stomodaeum. *sv*. Sinus venosus. *t*. Thyroid body. *ta*. Truncus arteriosus. *tp*. Tuberculum posterius. *v*. Ventricle. *vc*. Inferior (posterior) vena cava.

cavity of the fore-brain, or *third ventricle*, by a pair of openings, the *foramina of Monro*. During the remainder of larval life the hemispheres continue to grow forward and their walls to thicken. Their anterior ends become slightly constricted away from the main portion of the hemispheres as the *olfactory lobes*. At first these are separate, but later they become fused. Thus at metamorphosis when the cerebrum is virtually mature, it comprises half of the entire brain. Furthermore, on account of this cerebral increase and the direction of the growth, the relative proportion of the parts of the brain is so altered that the cranial flexure appears to vanish. As a matter of fact, however, it is actually unchanged.

Somewhat after the first appearance of the cerebral rudiment, i.e., at about 9 mm., a change occurs in the antero-dorsal wall of the third ventricle just below and slightly in front of the epiphysis. The thin roof of this region becomes folded and hangs down into the cavity of the ventricle. Later these folds become very vascular, and are known as the *anterior choroid plexus* (Figs. 90; 91, B).

With the appearance of this final structure of the prosencephalon, it is possible further to subdivide this region as follows. Suppose a plane to be passed transversely through the third ventricle from the anterior side of the choroid plexus, to the anterior side of the optic recess between it and the torus transversus. The portion of the ventricle anterior to this plane is then termed the *telencephalon*, and the portion posterior to it, the *diencephalon*. On this basis it is evident that the cerebral hemispheres arise from the telencephalon and the anterior choroid plexus from the anterior part of the diencephalon.

Although the pituitary body, as already noted, is not strictly a part of the brain, its further history may best be described at this point. The backward growth of the anterior (stomodaeal) part of this organ continues, and at about the same time that the choroid plexus appears, it loses its connection with the stomodaeal ectoderm. At the same time it acquires a cavity, and presently becomes united with the posterior (infundibular) part of the hypophysis, which retains its connection with the brain through the hollow infundibular stalk. Later the posterior portion of the anterior part of the hypophysis becomes convoluted and tubular. As regards terminology, it is to be remembered that the actual positions of the above mentioned "parts" are reversed in all adult Amphibia so that the anterior or stomodaeal part is really behind the posterior or infundibular part. Lastly in this connection it is of interest to note that experiment has shown that neither the stomodaeal or in-



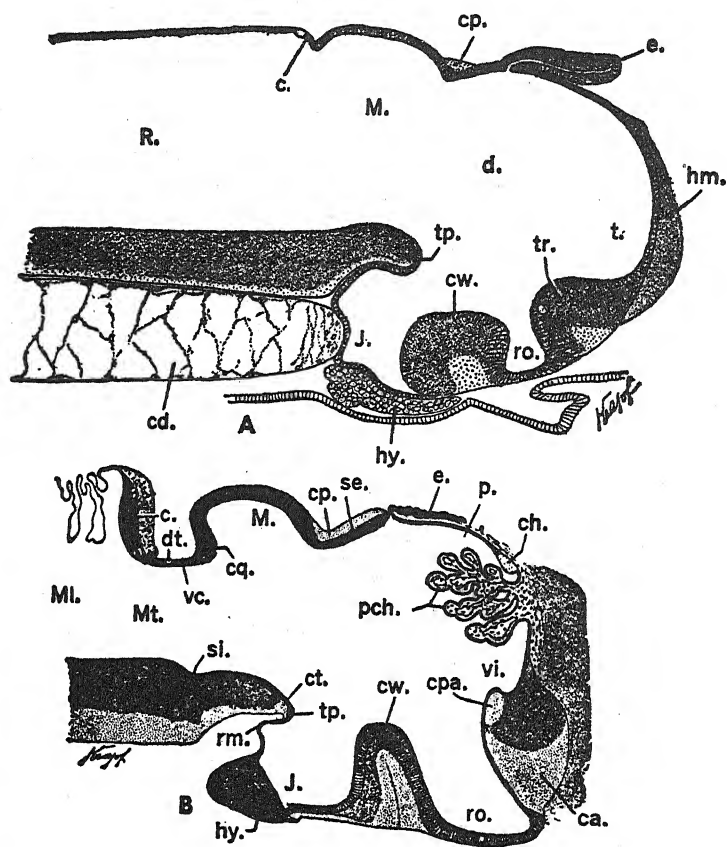


Fig. 91. — Median sagittal sections through the brain of the Frog. From Von Kupffer (Hertwig's *Handbuch*, etc.). A. Of a larva of *R. fusca* of 7 mm. in which the mouth was open. B. *R. esculenta* at the end of metamorphosis.

c. Cerebellum. ca. Anterior commissure. cd. Notochord. ch. Habenular commissure. cp. Posterior commissure. cpa. Anterior pallial commissure. cq. Posterior corpus quadrigeminum. ct. Tubercular commissure. cw. Optic chiasma. d. Diencephalon. dt. Tract of IV cranial nerve. e. Epiphysis. hm. Cerebral hemisphere. hy. Hypophysis (pituitary body). J. Infundibulum. M. Mesencephalon. Mt. Myelencephalon. Mt. Metencephalon. p. Antero-dorsal extension of diencephalon. pch. Choroid plexus of third ventricle. R. Rhombencephalon. rm. Recessus mammillaris. ro. Optic recess. se. Roof diencephalon. t. Telencephalon. tp. Tuberculum posterius. tr. Torus transversus (telencephali). vc. Valvula cerebelli. vi. Ventriculus impar (telencephali) (third ventricle).

fundibular part develops normally in the absence of the other (Smith, '20).

The Mesencephalon.—The structures of the mesencephalon or mid-brain are not so numerous as are those of the fore-brain. Its chief features are the *crura cerebri* and the *optic lobes*. The former arise gradually as a pair of ventro-lateral thickenings composed of nerve fibers connecting this portion of the brain with the fore-brain. The latter, i.e., the optic lobes, appear at about 9 mm. as a pair of swellings in the dorso-lateral regions of the roof. They attain their full size at about the time of metamorphosis, and their complete development is apparently dependent on the presence of normally developing eyes (Kollros, '53). The cavity of the mid-brain serves to connect the cavities of the fore- and hind-brains, and is termed the *aqueduct of Sylvius*.

The Rhombencephalon.—The rhombencephalon or hind-brain includes the *metencephalon* and the *medulla oblongata*. The principal development of the metencephalon immediately behind the mid-brain is quite limited in the Frog, the most prominent part being its roof which at about 9 mm. gives rise to a thickened transverse ridge, the *cerebellum* (Fig. 91). The medulla, on the other hand, is more extensive with a thin roof. The latter always remains thin but at the same time that the cerebellum starts to develop it begins to become folded. Soon blood vessels extend down into these folds, and thus is formed the *posterior choroid plexus* (Fig. 90, B). The floor and the ventro-lateral walls of the hind-brain become thickened as nerve tracts. Its cavity connecting anteriorly by way of the aqueduct of Sylvius with the third ventricle, and posteriorly with the neural canal, is called the *fourth ventricle*.

The Spinal Cord.—Posterior to the brain region the neural tube gradually assumes the character of the adult spinal cord. The laterally compressed neural canal is, as already noted, lined by cells which were originally external. These are non-nervous and ciliated, and are known as *ependymal cells*. The relatively thick nervous layer which constitutes the bulk of the lateral walls gives rise to both supporting or *glia* cells, and to *neuroblasts* or primitive nerve cells. The latter lie relatively near the central canal, and comprise the so-called *gray matter*. The fibers which arise from them, however, course up and down through the more superficial parts of the cord, helping still further to thicken it, and constituting the *white matter*.

This thickening occurs first in the dorsal-lateral regions, thereby causing the neural canal to lie temporarily very near to the ventral side

182 THE FROG: LATER OR LARVAL DEVELOPMENT

(Fig. 92, *A*). Gradually, however, the growth of cells and fibers spreads downward so that eventually the canal lies practically in the middle of the cord. The ventro-lateral growth, moreover, is slightly greater than

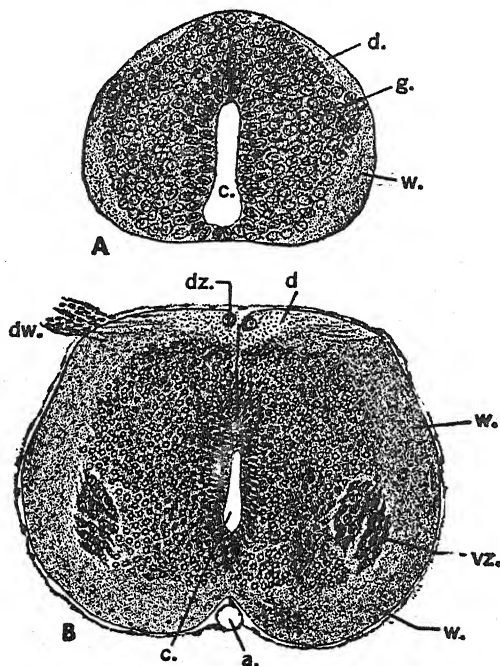


Fig. 92.—Transverse sections through the spinal cord of *R. fusca*. From Von Kupffer (Hertwig's *Handbuch*, etc.). *A*. Through the anal region of a larva of 7 mm. *B*. Through the anterior body region of a larva during metamorphosis.

a. Spinal artery. *c*. Central (neural) canal. *d*. Dorsal column (white matter). *dw*. Dorsal root of spinal nerve. *dz*. Atrophied dorsal cells. *g*. Gray matter. *vz*. Ventral cells. *w*. Dorso-lateral and ventro-lateral column (white matter).

that exactly along the mid-ventral line. Thus a shallow depression occurs here in which runs the spinal artery (Fig. 92, *B*).

Posteriorly the neurenteric canal becomes severed even before hatching, and the nerve cord continues straight out into the tail. This portion of the cord is of course lost at metamorphosis.

THE PERIPHERAL NERVOUS SYSTEM

The Cranial Nerves.—In discussing nerves in general, it is quite customary to divide them into *afferent* or sensory nerves, and *efferent*

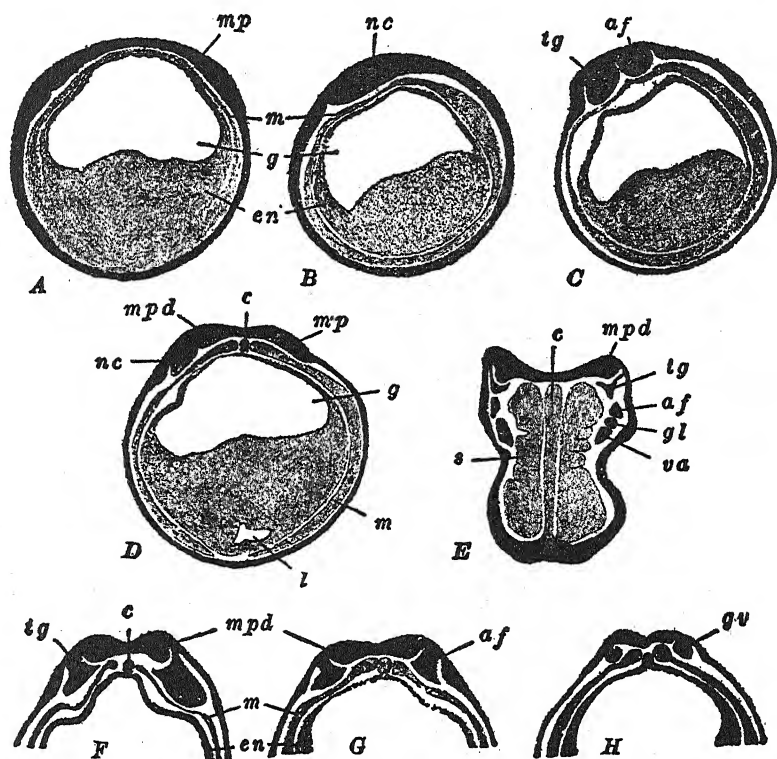


Fig. 93. — Sections through young Frog embryos (*R. fusca*), illustrating the development of the crest segments ("ganglia") and placodes. From Kellicott (*Chordate Development*). After Brachet. A. Transverse section through the neural plate of an embryo before elongation begins. B. Sagittal section to one side of the mid-line, through an embryo of the same age as A. C. Sagittal section, to one side of the mid-line, through an embryo just beginning to elongate. D. Transverse section through an embryo slightly older than that of A and B. E. Frontal section through an embryo with three or four pairs of mesodermal somites. F, G, H. Three transverse sections through an embryo just beginning to elongate (same age as C), showing the trigeminal, acustico-facial and glossopharyngeal-vagus crest segments ("ganglia").

af. Acustico-facialis crest segment ("ganglion"). c. Notochord. en. Endoderm. g. Gut cavity. gl. Glossopharyngeal crest segment ("ganglion"). gv. Glossopharyngeal-vagus crest segment ("ganglion"). l. Liver diverticulum. m. Mesoderm. mp. Primitive medullary plate. mpd. Definitive medullary plate. nc. Neural crest. s. Mesodermal somites. tg. Trigeminal crest segment ("ganglion"). va. vagus (pneumogastric) crest segment ("ganglion").

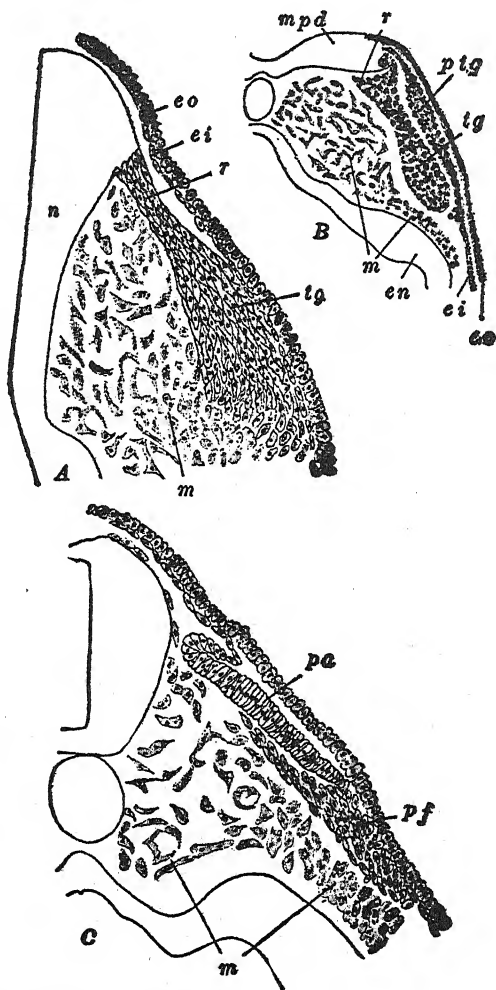


Fig. 94. — Portions of sections through the head of the Frog (*R. fusca*), illustrating the formation of the placodes and the history of the crest segments ("ganglia"). From Kellicott (*Chordate Development*). After Brachet. A. Transverse section through the trigeminal crest segment ("ganglion") of an embryo of 3 mm. B. Transverse section through the trigeminal crest segment ("ganglion") and placode of an embryo with three or four pairs of mesodermal somites. C. Transverse section through the facial ganglion and auditory placode of an embryo of 2.8 mm.
ei. Inner or nervous layer of ectoderm. *en*. Endoderm. *eo*. Outer layer of ectoderm. *m*. Mesoderm. *mpd*. Definitive medullary plate. *n*. Nerve cord. *pa*. Auditory placode. *pf*. Facial placode. *ptg*. Trigeminal placode. *r*. Spinal prolongation of ganglion. *tg*. Trigeminal crest segment ("ganglion").

or motor nerves. In describing both the cranial and spinal nerves, however, it is convenient to add a third category, i.e., *mixed nerves*, which contain both afferent and efferent fibers. It is understood that all these nerves occur in pairs, but it will be necessary to describe the development only on one side.

Purely Afferent Nerves. — There are three cranial nerves which are purely afferent; namely, the I or *olfactory nerve*, the II or *optic nerve*, and the VIII or *auditory nerve*. The first two are of a rather special nature, and are also very closely connected with the development of the sense organs which they supply. It will therefore be more convenient to describe them later in connection with those organs. The VIII nerve on the other hand arises in such close connection with the mixed nerves that it will be described under that category.

Mixed Nerves and the Auditory Nerve. — The nature of the neural crests has already been indicated, and it was noted that each crest becomes divided into segments. In the brain region there are three such segments on each side of the head. Considerably before hatching (3–4 mm.), moreover, the nervous layer of ectoderm on the inside of the head opposite the segments becomes thickened into patches termed *placodes*, one opposite each of the first two segments, and two opposite the last. It is then from certain nervous or ganglionic elements of these structures, i.e., the crest segments and placodes, that the ganglia of the V, VII, VIII, IX, and X nerves (Fig. 93) and their afferent fibers develop in the manner indicated below. The efferent fiber origins of all mixed nerves will be noted separately. It remains to state that the strands of cells attaching the crest segments to the brain merely contribute to the sheaths of the nerves whose origins are being described.

The V or *trigeminal nerve ganglion* develops from dorsal and superficial cells (the ganglionic element) of the most anterior crest segment along with cells derived from the inner or ganglionic portion of the corresponding placode (Fig. 94, B). The anterior part of the ganglion arises almost entirely from the anterior portion of the placode, and produces the afferent fibers of the *ophthalmic* branch of the V nerve. The posterior part consists of both crest and placode elements, and is sometimes distinguished as the trigeminal ganglion proper, or *Gasserian ganglion*. This part produces the afferent fibers of the *maxillary nerve* which are derived from the placodal element, and afferent fibers of the *mandibular nerve* which seem to come from the crest element (Knouff, '27). From both parts of the ganglion a common bundle of fibers also grows inward to the medulla constituting the sensory element of the V nerve

186 THE FROG: LATER OR LARVAL DEVELOPMENT

root. The ophthalmic branch of the nerve is destined for the skin of the snout, while the mandibular and maxillary branches supply the lower and upper jaw. As all of these branches start to develop previous to hatching, in a 9 mm. tadpole they are well established. It may be added that the non-nervous part of the crest segment, in this instance the major part, grows ventrally and contributes to the mesenchyme of the mandibular arch. The superficial (outer) non-nervous part of the placode, on the other hand, disappears.

It is now believed that the sensory elements of the VII or facial ganglion and nerve come exclusively from the second placode, while the sheath cells are both crest and placodal in origin. At least this has been proven for *Amblystoma* (Yntema, '37), and seems likely to be true also in the Frog. As before, some of the fibers which issue from this ganglion proceed inward to the medulla, forming the sensory element of the root, while others grow outward as the afferent fibers of the nerve. Before hatching, the latter have divided into the *hyoid* and *palatine* branches. Here also the considerable non-nervous part of the crest contributes in this case to the mesenchyme of the hyoid arch. No part of the placode in this instance, however, disappears. One portion is utilized as just described, while the remainder goes to form the ganglion of the VIII nerve and the auditory apparatus, as indicated below.

The IX and X or *glossopharyngeal* and *vagus* (pneumogastric) ganglia arise from the ganglionic portion of the last cranial crest segment in conjunction with the inner, i.e., ganglionic part, of the third and fourth placodes respectively. In these cases both crest and placode contribute neurons as well as sheath cells. Fibers from these two ganglia enter the medulla as a single root. Peripheral outgrowths from the IX ganglion supply the first branchial arch, while branches from the X pass to the remaining branchial arches. The vagus ganglion also sends branches to the viscera and to the lateral line organs (see below), the nerves to these parts being entirely placodal in origin. At least this appears true for *Amblystoma* (Yntema, '43), the situation in the Frog not having been so extensively investigated. Both of these ganglia with their nerves develop quite early, and in a 9 mm. larva all the main branches of the vagus nerve are present. In this case the non-nervous part of the crest segment is not large, but, so far as it exists, it goes to form mesenchyme. The superficial non-nervous portions of the placodes disappear.

It may now be added that the efferent fibers (axones) for each of these four nerves (V, VII, IX, and X) grow out from neuroblasts in the

walls of the medulla. They pass out of the brain along with the sensory root fibers of the respective ganglia, and having passed through these ganglia they enter the outgoing branches of the mixed cranial nerves.

The VIII or *auditory nerve* is, as already noted, entirely sensory, and its ganglion arises from the ganglionic portion of that part of the second placode which is not involved in the formation of the ganglion of the VII nerve. The more superficial portion of this placode as usual is not included in either the VII or VIII nerve ganglion, but nevertheless, as suggested above, it does not in this instance disappear. Instead it remains in close contact with the latter ganglion, and develops later into the so-called inner ear, as described below. Because of the prominent part which the major portion of this second placode then plays in connection with the auditory apparatus, it is frequently referred to as the *auditory placode* (Fig. 94, C), already noted in the account of an earlier stage (Camphenhout, '35). The roots of the VII and VIII nerves are indistinguishable from one another previous to the opening of the mouth (9 mm.).

Purely Efferent Nerves.—The III, IV, and VI nerves are all *motor ocular* nerves which innervate the muscles of the eye. Their development is imperfectly known, but they seem to arise from neuroblasts in the mid-brain and medulla. The III appears first, just before hatching, the others slightly later.

The Spinal Nerves.—The ganglia of the spinal nerves, unlike those of the cranial nerves, arise entirely from the neural crests, no placode elements in this case being involved. The division of the originally continuous crests of this region into the segments which eventu-

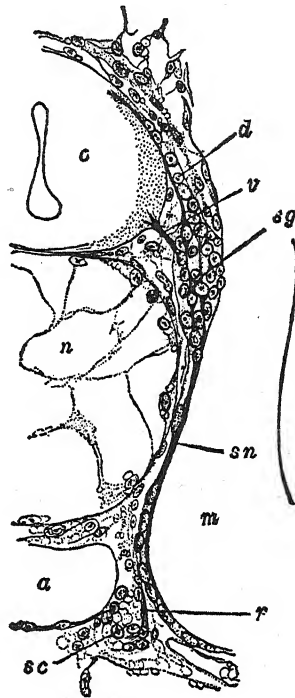


Fig. 95.—Transverse section through 8.6 mm. larva of *R. esculenta*, illustrating the relations of the sympathetic cord and spinal nerve. From Kelliecott (*Chordate Development*). After Held.

a. Dorsal aorta, c. Spinal cord. d. Dorsal (sensory, afferent) root of spinal nerve. m. Myotome. n. Notochord. r. Ramus communicans. sc. Sympathetic cord. sg. Spinal ganglion. sn. Spinal nerve trunk. v. Ventral (motor, efferent) root of spinal nerve.

ally become the ganglia is apparently conditioned, moreover, by the previous segmentation of the myotomes (Lehman, '27, Detwiler, '37). Also if more or fewer myotomes are experimentally produced the related ganglia are correspondingly increased or decreased in number (Detwiler, '34). From each crest segment, fibers grow inward and connect with the dorsal part of the cord. These are known as the *dorsal roots* of the spinal nerves (Fig. 95). At the same time other fibers grow outward to the skin, and other sensory organs; as in the head, all of these ganglion fibers are afferent.

While this is occurring dorsally *ventral nerve roots* also arise (about 4 mm.). Each of these roots consists of a bundle of fibers (*axones*) originating from neuroblasts in the ventral part of the spinal cord. This has been confirmed experimentally by removing parts of the cord while leaving the crests, in which case the ventral roots are absent (Taylor, '44).

At or just beyond each dorsal root ganglion the fibers of the respective ventral bundle mingle in a common sheath with the outgoing fibers of the ganglion. Thus, since the ventral root fibers are all efferent, each nerve sheath containing both sorts constitutes a mixed nerve (*spinal nerve trunk*) as in the cases of a similar condition in the head. This trunk soon divides into a dorsal and a ventral branch, each of which now contains both afferent and efferent fibers; the former pass to the various sense organs and the latter to the muscles.

The problem of how these and other fibers are directed to their proper destinations has long been of interest, and is not yet completely solved. There does appear to be a tendency, however, for outgrowing nerves to proceed toward certain kinds of tissue more than toward others. Thus Detwiler ('36) has shown that whereas transplanted pieces of brain failed to attract such nerves, transplanted limb, eye, and nasal placode do so in the order indicated. Even so the attraction is apparently not very specific, i.e., certain nerves are not inevitably attracted to their normal muscles, as shown by somewhat displacing the sources of the nerves (Piatt on *Amblystoma*, '40). The nature of such general attraction as there may be is not known, but may be tentatively assumed to be both mechanical and chemical in character. Finally it may be noted that there is also a question as to what causes more anterior parts of the spinal cord to contain more nerve cells than the relatively caudal parts. There has been some evidence that what a given segment contains is dependent to some extent on the character of the part anterior to it. Thus if a piece of spinal cord were substituted for the medulla this might be expected to lead to fewer cells and fibers in the cord posterior to the

implant. Such, however, seems not to be true in this case, thus suggesting, to a certain degree at least, an inherent developmental capacity in various levels of the cord (Detwiler on *Amblystoma*, '37).

The Sympathetic System.—In the sympathetic system the neuroblasts have been shown to originate both from the neural crests and the neural tube, while the sheath cells come entirely from the latter. At least this has been demonstrated experimentally for *Triton* by replacing part of its neural tube by easily distinguishable material from *Axolotl* (Ra-

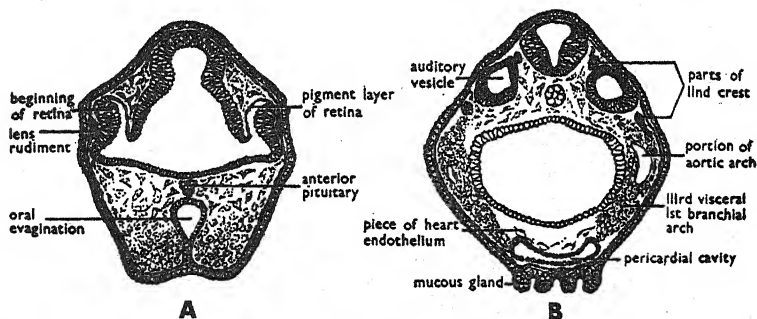


Fig. 96. — Cross sections of 4 mm. stage of Frog embryo. *A*. Section through the optic cups starting to form the vesicles. *B*. Section through the auditory vesicles and extreme anterior of the heart rudiment. This section also passes through the pharyngeal region at the level of the third visceral or 1st branchial arch.

ven, '36), and it is probably true of other Amphibia. The cells of the sympathetic system first appear, however, in small collections upon the spinal nerves at about the level of the dorsal aorta, a position in which they may be noted shortly before hatching. Presently they migrate to the aorta, along each side of which they give rise to a *sympathetic cord*. From these cords, nerve fibers later grow back to the spinal ganglia, as the *rami communicantes*. Still other fibers proceed to the viscera, and along these, cells migrate to form the various peripheral sympathetic ganglia.

ORGANS OF SPECIAL SENSE

The Eye.—When the rudiments of the eye were last considered the optic stalks were just beginning to be defined as such, owing to a slight constriction between the optic vesicles and the brain. This process is now rapidly completed so that the stalks are clearly indicated. It is then evident that they do not join the vesicles exactly at the centers of the latter but nearer to their ventral sides. There then begin certain changes in connection with the vesicles themselves as follows:

The wall of each vesicle next to the ectoderm is flattened and then pushed inward. By this process the cavity of the optic vesicle is obliterated, and at the same time a double-walled cup is formed, the *optic cup* (Figs. 96, 97, 98). It must be noted, however, that the direction of this invagination is not exactly horizontal. It begins rather in the ventro-lateral region and proceeds obliquely upward. This fact, together with

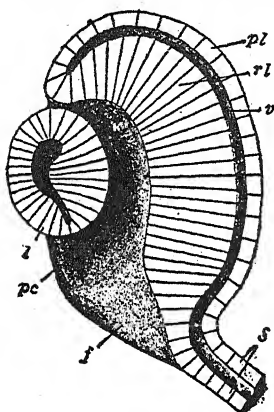


Fig. 97.—Plastic figure of hemisected optic vesicle, lens and optic stalk of the Frog. From Kellicott (*Chordate Development*).

f. Choroid-fissure. *l.* Lens. *pc.* Posterior (Vitreous) chamber of eye. *pl.* Outer or pigmented layer of optic cup. *rl.* Inner or retinal layer of optic cup. *s.* Optic stalk. *v.* Original cavity of optic vesicle.

the original relation of the vesicle and stalk, means that the latter will necessarily be attached to the cup at its ventral edge. The rim of the cup now grows outward, particularly in its ventral and lateral regions, these being the regions which, as a result of the direction of invagination, are further from the ectoderm. This outward extension of the sides of the cup leaves between their ventral edges a slight fissure extending inward to the optic stalk. This is the *choroid fissure*, whose length is somewhat further increased by the continued outgrowth of the sides of the cup. Furthermore, concurrent with this outgrowth the entire rim begins to bend toward the center of the cup's aperture, thus obviously decreasing its diameter. This aperture, which faces the ectoderm, is the *pupil*, from whose ventral edge the choroid fissure runs back to the optic stalk.

Meanwhile, about the time of hatching, a thickened portion of the inner ectoderm on the wall of the head opposite the pupil becomes constricted off as a solid rounded mass of cells (Fig. 98). This is sometimes, though erroneously, called the visual placode. It presently acquires a central cavity, which is soon obliterated, however, by the thickening of the cells on the future retinal side. This mass now moves in to the center of the pupil, and becomes the *lens*. The invagination of the ectoderm to form the lens appears to be induced by the adjacent optic vesicle (Beckwith, '27), though the competence of all ectoderm so to respond has been questioned. Thus, in this as in some other cases, the ability of ectoderm to react specifically at a given stage seems to depend upon its earlier subjection to another inductive agent, e.g., the mesentoderm (Liedke, '51). On the other hand, under

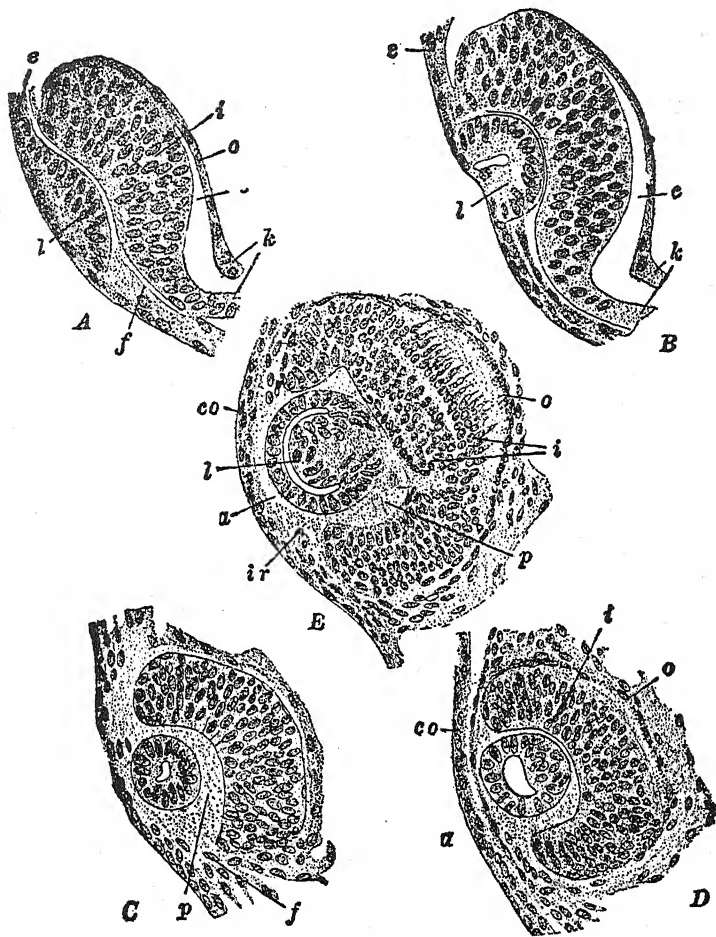


Fig. 98. — The development of the eye in the Urodele, *Siredon pisciformis*. From Kellicott (*Chordate Development*). After Rabl. A. Of embryo with about twenty-five pairs of somites, showing the thickening of the lens rudiment. B. Invagination of the lens and formation of the optic cup. C. Lens separating from the superficial ectoderm in an embryo of about thirty-five pairs of somites. D. Thickening of the inner wall of the lens. E. Shortly before hatching; differentiation of the rods and cones in the retinal layer. *a*. Anterior chamber of eye. *c*. Cavity of primary optic vesicle. *co*. Cornea. *e*. Ectoderm of head. *f*. Choroid fissure. *i*. Inner or retinal layer of optic cup. *ir*. Rudiment of iris. *k*. Optic stalk. *l*. Lens. *o*. Outer or pigmented layer of optic cup. *p*. Posterior (vitreous) chamber of eye.

certain conditions it is known that if at the neural fold stage a lens has been removed it can only be replaced by cells derived from the dorsal rim of the iris (see below).

Shortly after hatching the cells in the walls of the optic cup begin to differentiate. The inner wall thickens and develops into the *retina*, its outermost cells becoming the *rods* and *cones*. Its inner cells, i.e., those toward its cavity, form neuroblasts which send axones over the inner surface just beneath the thin *internal limiting membrane*, which is produced from fibers growing out from non-nervous cells deeper in the retina. The axones, leaving the cup through the inner end of the choroid fissure, grow within the substance of the ventral wall of the optic stalk to the brain, where those from opposite sides cross to form the *optic chiasma*. The ventral wall of the stalk, thickened by its axones, soon obliterates the stalk lumen, the other stalk cells disappear, and the neural sheath is formed of connective tissue, the axones and sheath cells together constituting the II or *optic nerve*. The outer wall of the cup adjacent to the rods and cones develops pigment, and hence is called the *pigment layer* of the retina.

Slightly before hatching the lips of the choroid fissure begin to fuse, and shortly this fusion becomes complete everywhere except next to the optic stalk, where the blood vessels and axones leave the cavity of the cup. At the edge of the pupil the closure is marked by a thickening, the *choroid knot*, from which arise the cells of the iris. This closure of the fissure is said not to occur in the absence of the lens (Beckwith, '27).

The *vitreous humor* is formed in the cavity of the cup by cells budded from the retinal wall and from the inner side of the lens. It is thus entirely ectodermal. The *choroid coat* of the eye is laid down outside the pigmented layer, and outside of all is the tough *sclerotic coat*. Both the choroid and sclerotic tissues are derived from mesenchyme. Opposite the lens the ectoderm of the head becomes transparent, and, again with the addition of mesenchyme, forms the *cornea*. The detailed development of the eye is not entirely completed until metamorphosis.

The Ear.

The Inner Ear or Membranous Labyrinth.—Just before hatching the superficial part of the auditory placode, i.e., the part not involved in the formation of the VII and VIII nerve ganglia, moves in slightly from the ectoderm. At the same time it invaginates to form a closed membranous vesicle, the *auditory sac* or *otocyst*. By appropriate transplantations it was shown that the differentiation of this sac is induced

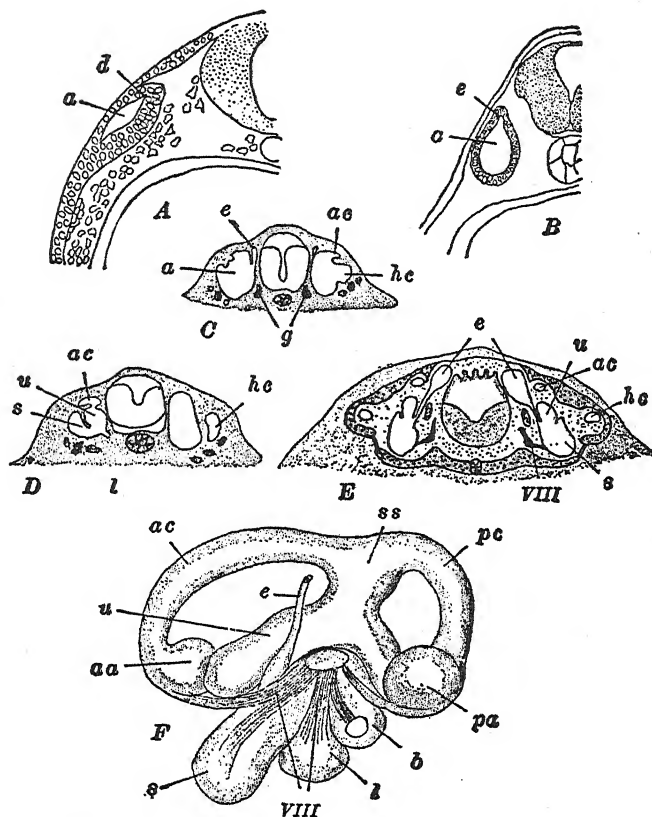


Fig. 99. — The development of the auditory organ in the Frog and Toad. From Kellicott (*Chordate Development*). A, B, F. After Krause. C, D, E. After Villy. A. Section through the auditory vesicle of an embryo just beginning to elongate. B. Section through the auditory vesicle that has very nearly separated from the superficial ectoderm. C. Transverse section, somewhat oblique, through the auditory organs of a 12 mm. *R. temporaria*. D. Slightly more advanced stage than C. E. Section through the auditory organs of a 25 mm. *R. temporaria*. F. Membranous labyrinth of the Toad (*Bufo vulgaris*).

a. Auditory sac. aa. Anterior ampulla. ac. Anterior vertical semicircular canal. b. Pars basilaris. d. Dorsal outgrowth of primitive auditory vesicle (rudiment of endolymphatic duct). e. Endolymphatic duct. g. Ganglion of auditory (VIII) nerve. hc. Horizontal semicircular canal. l. Lagena or cochlea. pa. Posterior ampulla. pc. Posterior vertical semicircular canal. s. Sacculi. ss. Sinus superior. u. Utricle. VIII. Auditory nerve.

by the presence of the medulla, and also to some extent by the roof of the archenteron. This seems to be true even when the medulla is from a different species of Amphibian. As in the case of lens induction, however, it again appears that ectoderm near the normal site is more competent to respond in this manner than that from elsewhere (Albaum and Nestler, '37, and Zwilling, '41). From the dorsal wall of the otocyst a small evagination now appears which is the rudiment of the *endolymphatic duct* (Fig. 99, A, B). An oblique partition then (10–12 mm.) begins to grow across the cavity of the otocyst in such a way as to divide it into a lateral and ventral portion, the *sacculle*, and an upper and median portion, the *utricle*. These cavities remain connected by a small pore in the membrane (Fig. 99, D).

During the growth of the above partition there appear upon the inner surface of the wall of the utricular portion of the otocyst, two pairs of ridges. One pair is vertical and anterior, the other horizontal and lateral, upon the side nearest the ectoderm. Presently (15 mm.), there is added another pair which is posterior and vertical. The edges of each pair of ridges now fuse with one another along their entire length, thus giving rise in each case to a tube open at each end into the cavity of the utricle. The tubes thus formed are the rudiments of the three *semi-circular canals*. From the manner of their formation these tubes or canals evidently lie upon the inside of the utricular wall. Shortly, however, each canal pushes outward and presently becomes constricted away from the wall of the utricle except at its ends. The canals which thus come to lie outside of the utricle now continue to grow, and so reach the adult condition. During this latter process, however, each canal acquires an enlargement at one of its ends termed an *ampulla*. These ampullae are not developed from the canals themselves, but are added to them through a further constricting off of portions of the utricle (Fig. 99, E, F).

Meanwhile the sacculle in the course of its separation from the utricle has become the part of the otocyst which receives the endolymphatic duct. The two ducts, one from each side of the head, then grow up over the brain; during this process their ends become enlarged (at about 20 mm.) to form the *endolymphatic sacs*. By the time of metamorphosis, these sacs have increased greatly in size, have become very vascular, and fused with each other. In the adult they form a considerable vascular covering for the myelencephalon. It is also stated by Wilder ('09) that in all the Anura an outgrowth from each endolymphatic sac extends down along the side of the dorsal nerve cord outside the dura

mater. Where each spinal nerve root emerges an extension from these outgrowths also emerges, and forms a small pocket partially wrapped around the respective spinal ganglion. These pockets are filled with calcareous material, and it is this whitish substance seen through the pocket wall that one observes when viewing the "ganglia" in a gross dissection of the Frog.

In larvae of 15-20 mm. the sacculæ is also giving rise to two other structures as follows: From its upper portion the *lagena* or *cochlea* arises as a postero-ventral evagination, while a similar and slightly more dorsal outpushing, in close connection with the first, constitutes the *basilar chamber* (*pars basilaris*) (Fig. 99, F).

Sensory patches develop on the inside of the epithelial walls of the utricle, sacculæ, cochlea, and ampullae, and these are connected with branches of the auditory nerve which proceeds from its ganglion. The entire membranous labyrinth thus formed is eventually encased in cartilage and bone arising from the surrounding mesenchyme. The casing follows the contour of the membrane, and constitutes the *auditory capsule*. There is a slight space between the capsule and membrane, the *perilymphatic space*, and this is filled with *perilymphatic fluid*.

At this point experimental procedures have again been applied which show that not only is the membranous otocyst produced by induction, but that it in turn induces the formation of the cartilaginous capsule around it (Kaan, '38). Apparently not quite any mesoderm is competent to react in this way, but at least that of the head region and some of the somites will do so. Kaan also noted a reciprocal action in that a normal capsule was necessary to induce the membranous otocyst to go on and develop a normal membranous labyrinth. Thus we see a good illustration of the continuous actions and reactions in a developmental system that has once been set going.

The Middle Ear.—This portion of the auditory organ develops chiefly during and after metamorphosis, as follows: The vestigial visceral pouch between the mandibular and hyoid arches, i.e., the hyomandibular, produces from its dorsal end a rod of cells with a terminal knob. This rod grows out until the knob reaches a position between the inner ear and the wall of the head. A cavity then develops in the knob and in the rod of cells. The cavity in the knob is the *tympanic cavity*, and that in the rod the *Eustachian tube*, which connects the cavity with the pharynx. The tympanic cavity, or cavity of the middle ear, increases in size until its outer wall fuses with the ectoderm. The membrane thus formed is the *tympanic membrane* or ear drum, separating the tympanic

cavity from the exterior. This membrane it may be noted has a special histological character, and Helff ('28) has proven that this character is induced by the presence of two pieces of cartilage. One is the *annulus tympanicus*, a ring-shaped structure which surrounds the membrane at its periphery and supports it. The other will be indicated presently. Helff ('34) has shown further that rings of cartilage cut from the supra scapula have a slight tendency to produce changes in the ectoderm similar to those produced by the annulus tympanicus. He has also shown that rings cut from the palato-quadrate cartilage (see account of skeleton) will act just as well as the annulus tympanicus itself. This last fact is significant for the following reasons: In the lower Vertebrates the palato-quadrate forms a part of the upper jaw, and it has long been suspected that a small part of it survives in the higher members of this group as a bone of the middle ear. Such a hypothesis is obviously strengthened by this observation of the similar peculiar inductive qualities possessed by both palato-quadrate and annulus tympanicus.

Continuing with the history of the middle ear, we find that opposite to the tympanic membrane the wall of the tympanic cavity contacts the auditory capsule. Here there is an aperture in the latter, the *fenestra ovalis*, opening into the perilymphatic space. In this aperture there develops a cartilaginous plug, the *operculum*. Across the roof of the tympanic cavity there is also formed a cartilaginous rod connecting the operculum with the tympanic membrane. It is the plectrum or columella, and is thought to be a vestige of the upper part of the hyoid arch. It will be recalled that the histological character of the tympanic membrane is due to two pieces of cartilage one of which is the annulus tympanicus. The other is the columella, without which the peculiar yellow fibers of the membrane are not formed (Helff, '31). Finally, at the close of metamorphosis, the columella separates from the dorsal wall of the tympanic cavity, so that it stretches freely from the tympanic membrane to the operculum. The columella and operculum then fuse, and the latter and part of the former become ossified. Interestingly enough in the larvae of some Frogs a temporary so-called bronchial columella connects the inner ear and the lung (Witschi, '55). This is suggestive of the ossicles connecting the air bladder and the inner ear in some Fish.

There is no *outer ear*, the tympanic membranes appearing on the outside of the Frog's head.

The Olfactory Organ.—In the account of the external developments, we have already referred to the olfactory pits, which are evident, even in a 2.5 mm. larva. Each is situated slightly above and anterior to

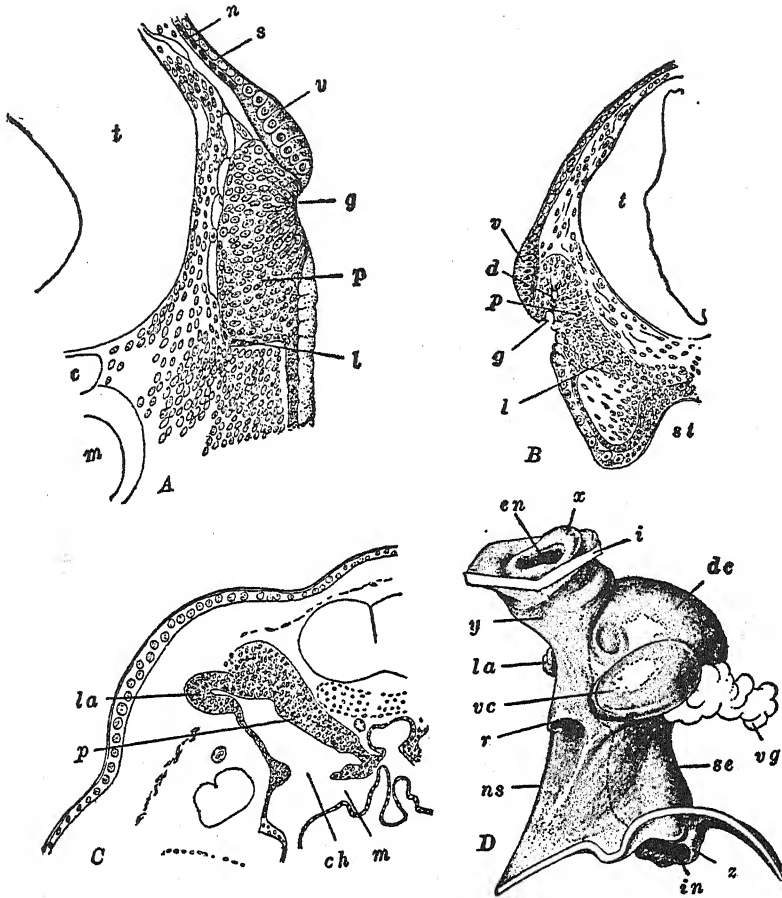


Fig. 100. — The development of the olfactory organ in *R. fusca*. From Kellicott (*Chordate Development*). After Hinsberg. A, B, C. Sections through the olfactory pit and organ of 5 mm., 6 mm., and 11 mm. larvae, respectively. D. Medial view of a model of the olfactory organ of a 31 mm. larva. The dotted line marks the limit between the sensory and non-sensory portions of the epithelial lining of the olfactory cavities.

c. Hypophysis. ch. Internal nares (choanae). d. Dorsal lumen. dc. Dorsal sac. en. External nares. g. Olfactory pit. i. Cut edge of integument. in. Internal nares (choanae). l. Elongation toward the mouth. la. Lateral appendix. m. Mouth cavity. n. Inner or nervous layer of ectoderm. ns. Part of chamber lined with non-sensory epithelium. p. Olfactory placode. r. Ridge marking the limit between middle and ventral chambers. s. Superficial layer of ectoderm. se. Part of the chamber lined with sensory epithelium. st. Stomodaeum. t. Telencephalon. v. Thickened bands of superficial ectoderm cells (possibly the vestige of a primitive sense organ). vc. Ventral sac. vg. Ventral nasal gland attached to Jacobson's organ. x. Elevation around external nares. y. Canal leading to olfactory cavity. z. Fold around internal narial opening.

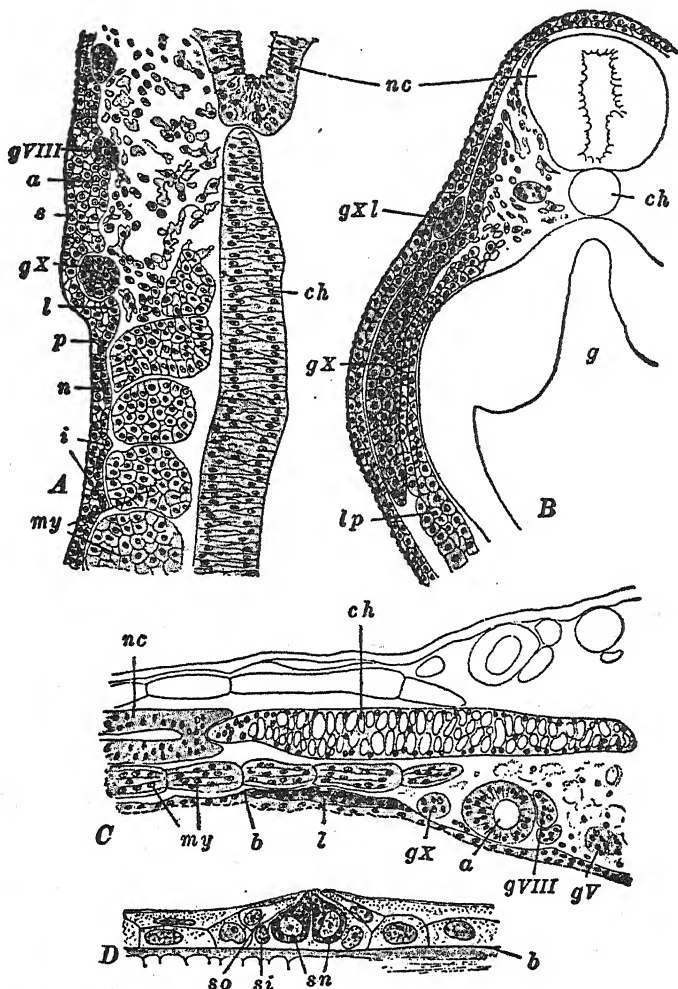


Fig. 101. — Development of lateral line organs in *R. sylvatica*. From Kellicott (*Chordate Development*). After Harrison. *A*. Part of a frontal section through level of notochord of a 3.3 mm. embryo. *B*. Part of a transverse section through vagus region of a 4 mm. embryo. *C*. Part of a frontal section through a 4 mm. embryo of *R. virescens*. *D*. Section through lateral line organ of a 15.5 mm. larva of *R. sylvatica*.

a. Auditory vesicle (in *A*, its rudiment). *b*. Basement membrane of epidermis. *ch*. Notochord. *g*. Gut. *gV*. Trigeminal ganglion, of V cranial nerve. *gVIII*. Acoustic ganglion of VIII cranial nerve. *gXI*. Ganglion of lateral nerve (branch of the vagus). *i*. Intersegmental thickenings of epidermis (ectoderm). *l*. Rudiment of lateral line nerve. *lp*. Lateral plate of mesoderm. *my*. Myotomes. *n*. Inner or nervous layer of epidermis (ectoderm). *nc*. Nerve cord. *p*. Pigment in epidermis. *s*. Superficial layer of epidermis (ectoderm). *si*. Inner sheath cells of lateral line organ. *sn*. Sensory cells of lateral line organ. *so*. Outer sheath cells of lateral line organ.

the side of the mouth. As these pits form, the superficial epithelium in this case disappears, while the inner invaginating layer thickens. These thickenings, which thus constitute the walls of the pits, are the olfactory placodes already indicated (Figs. 83, 100). Compare with Figure 88 of the exterior for general location.

A little after hatching there grows inward and downward from the floor of each pit a solid rod of cells. These rods presently become connected with the buccal cavity just at the posterior limit of the stomodaeum, and in tadpoles of 12 mm., each has acquired a lumen. Their openings into the cavity thus constitute the *internal nares*.

Somewhat later the olfactory lobes develop from the cerebrum, as indicated above. From each of these lobes, cells are then proliferated, which mingle with other cells derived from the placodes. The two strings of tissue thus constituted seem to become the sheaths of the I or *olfactory nerves*. The actual fibers of these nerves, however, arise from neuroblasts in the placodes, and grow backward to the lobes.

Meanwhile the pits are enlarging as the *nasal cavities*, and the remainder of the placode cells line them as the nasal epithelium. In the course of growth the cavities are removed somewhat from the surface of the head, but remain connected with it by tubes whose outer openings form the *external nares*. Changes in the shape and the proportion of the head alter from time to time the direction of the olfactory tracts. Thus these tracts first become vertical rather than horizontal, and later during metamorphosis develop a sharp flexure, due to the backward movement of the internal nares. At this latter period, also, each of the nasal cavities becomes greatly modified by complex evaginations and foldings. Of the former the most prominent arises ventro-medially from each cavity. The two bodies thus produced are the *organs of Jacobson*; they later acquire glandular masses at their medial ends.

The Lateral Line Organs.—At about 4 mm., a small dorso-lateral portion of the vagus ganglion of each side separates from the remainder and unites with a part of the most posterior or fourth placode. The placode then grows backward through the epidermis until, just before hatching, it reaches the tip of the tail (Fig. 101). At intervals along this cord there meanwhile arise groups of sensory cells which push their way to the surface and develop hair-like processes. These organs are innervated by a branch from the X nerve ganglion constituting the *ramus lateralis* (*lateral line nerve*). Other similar sensory organs develop in rows on the head, and are innervated by branches of the VII, IX, and X nerves. All these organs disappear at metamorphosis.

INTERNAL DEVELOPMENT: THE ALIMENTARY CANAL AND DERIVATIVES

THE MOUTH

When last described, the endoderm in the antero-ventral part of the pharyngeal region of the fore-gut had pushed out an evagination toward the ectoderm. The ectoderm had also "pitted in" toward this evagination to form the stomodaeum already noted. The stomodaeal wall now meets and fuses with the endodermal wall in this region forming the *oral plate* or *oral membrane* (Fig. 90, A). A few days after hatching (about 9 mm.), the oral plate becomes perforated, and henceforth the stomodaeal cavity or mouth communicates freely with the pharynx. The margins of the small larval mouth are formed fundamentally of the *mandibular ridges*, i.e., the outer edges of the mandibular arches. Outside of these ridges, however, the skin is drawn forward to form the *dorsal* and *ventral lips*.

The dorsal lip of the larva soon develops three medially incomplete rows of "teeth." Each of these teeth is formed from a cornified ectodermal cell which is periodically replaced by a similar cell pushing up from beneath. The ventral lip has four rows of such teeth; these rows, however, are complete. At the base of each lip, parallel with the rows of teeth, is a hardened ridge or jaw, also formed of cornified ectoderm.

At metamorphosis the horny teeth and jaws are lost, the adult jaws being of course much wider than those of the larva and formed largely of elements derived from the mandibular arch (Marshall). The permanent teeth occur only on the upper jaw, and are similar in their general structure to mammalian teeth. The *tongue* develops at this time from a proliferation of cells in the floor of the pharynx.

THE FORE-GUT AND ITS DERIVATIVES

The Visceral Arches and Pouches. — The beginnings of the first three pairs of pouches arising as solid vertically elongated evaginations of endoderm have already been indicated. The most anterior pair are the rudiments of the hyomandibular pouches, whereas the second and third pairs are the rudiments of the first and second branchial pouches. There presently arise three more pairs of these solid rudiments, making in all six pairs, one hyomandibular and five branchial, the last pair, however, being mere vestiges. The condition of both pouches and arches at hatching may be summed up in the following manner (Fig. 102):

With the exception of the sixth and last, the pouch rudiments, as noted, push out until they finally reach and fuse with the ectoderm of the corresponding clefts. They thus divide the mesoderm into the following bars or visceral arches: (1) the mandibular arch in front of the first or hyomandibular pouch; (2) the hyoid arch between the hyomandibular pouch and the first branchial pouch; (3) the first branchial arch following the first branchial pouch; (4) the second branchial arch following the second branchial pouch; (5) the third branchial arch following the third branchial pouch; (6) the fourth branchial arch, poorly defined, and following the fourth branchial pouch. There are thus six arches in all, beginning with the mandibular arch in front of the hyomandibular pouch, and ending with the fourth branchial arch in front of the last vestigial fifth branchial pouch.

The further development of the gill slits and gills has already been partially described in the account of the exterior. Nevertheless, it will be well at this point to recall the main features indicated, and to add certain details.

It will be remembered that, at about the time the mouth opens, the pharynx was said to be placed in communication with the exterior by means of the four pairs of branchial clefts and pouches. The changes in the solid pouches which make this possible, however, remain to be noted. Shortly after hatching, cavities appear in the first four pairs of branchial pouches, and these cavities become continuous with that of the pharynx. The cavities of the second and third pairs of branchial pouches then acquire openings to the outside by breaking through the points of fusion between the invaginated ectoderm and the endoderm,

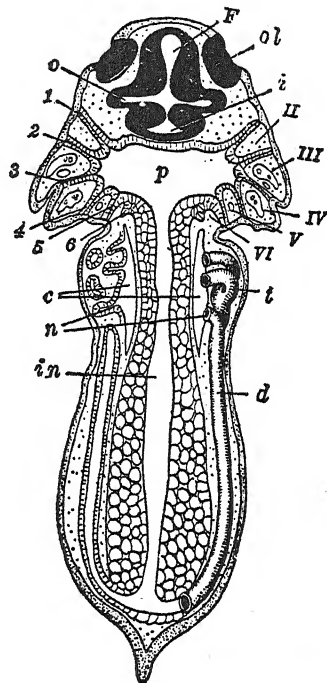


Fig. 102. — Diagram of a frontal section of a Frog larva at the time of hatching. From Kellicott (*Chordate Development*). After Marshall (modified). (*Vertebrate Embryology*, courtesy of Putnam's Sons.)

c. Coelom. d. Pronephric duct. F. Fore-brain. i. Infundibulum. in. Intestine. n. Nephrostome. o. Base of optic stalk. ol. Olfactory pit (placode). p. pharynx. t. Pronephric tubules. II. Hyoid arch. III-VI. First to fourth branchial arches. I. Hyomandibular pouch. 2-6. First to fifth branchial pouches.

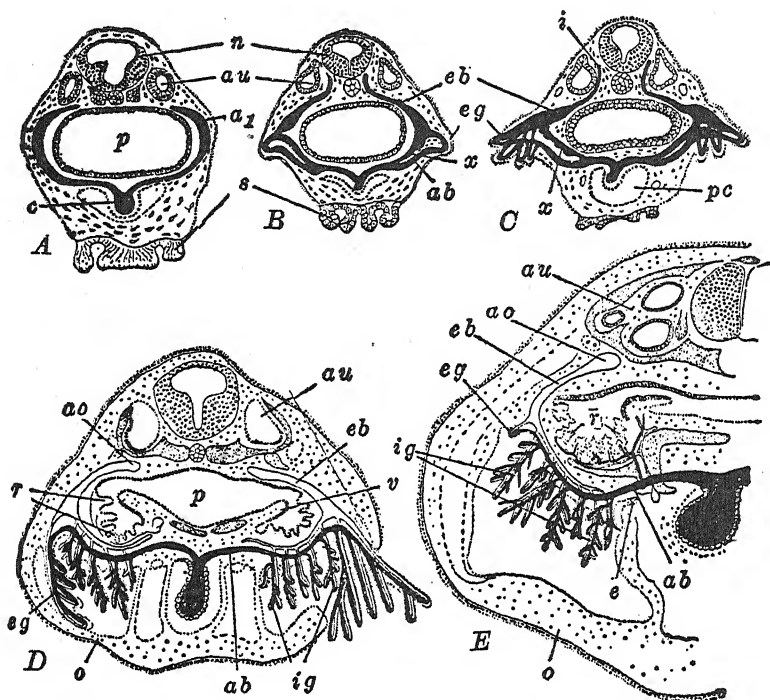


Fig. 103. — Semi-diagrammatic sections through the branchial region of tadpoles of *R. esculenta*, showing the development of the gills and the history of the aortic arches. From Kellicott (*Chordate Development*). After Maurer. A. 4 mm. larva showing the continuous first branchial aortic arch. B. 5 mm. larva showing the anastomosis between the afferent and efferent portions of the aortic arch. C. 6 mm. larva with vascular loops in the external gills. D. 13 mm. larva. On the left the opercular chamber is closed and the external gill is beginning to atrophy, while on the right this chamber is still open and the external gill well developed and projecting through the opercular opening. E. 17 mm. larva. Vessels of the second branchial arch. External gill represented only by a minute pigmented vestige.

a₁. First branchial aortic arch. ab. Afferent branchial artery. ao. Root of lateral dorsal aorta. au. Auditory organ. c. Conus arteriosus. e. Epithelioid body. eb. Efferent branchial artery. eg. External gill. i. Internal (anterior) carotid artery. ig. Internal gills. n. Nerve cord. o. Operculum. p. Pharynx. pc. Pericardial cavity. r. Gill rakers. s. Oral "sucker." v. Velar plate of floor, roof plates not visible here. x. Anastomosis between afferent and efferent branchial arteries.

and the cavities of the first and fourth presently do likewise. The two hyomandibular pouches never develop any real cavities, however, and the tissue which composes them later disappears. Since, likewise, there are no cavities in the fifth vestigial branchial pouches, there are formed altogether but four pairs of actual gill slits.

It has been noted that after the external gills are covered by the oper-

culum they soon atrophy and are functionally replaced by the *internal gills*. On the first three pairs of branchial arches these consist of a double row of filaments situated just ventral to those which are disappearing, but upon the posterior side of each arch, rather than upon its outer face. There is also a single row of filaments upon the anterior side of each of the fourth branchial arches. It is due to the fact that these new gills are upon the sides of the arches instead of upon their outer faces

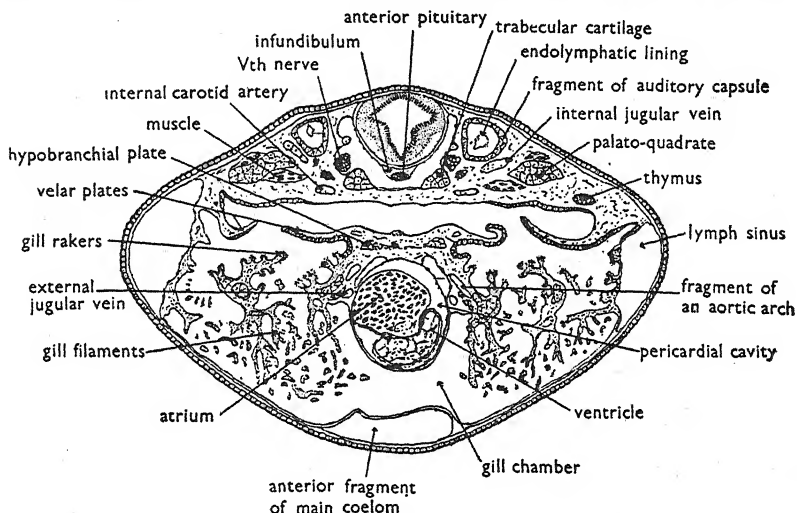


Fig. 104. — Cross section through the head of a late 10 mm. Frog larva in the region of parts of the 1st, 2nd, and 3rd branchial arches. The arches are cut transversely because of their diagonal courses. Only the extreme anterior portions of the auditory vesicles appear.

that they are termed internal. Nevertheless, they are still ectodermal rather than endodermal, and project well into the branchial (opercular) chamber. Thus, save for the fact that they are covered by the operculum, the term internal as applied to them is something of a misnomer. Meanwhile during the development of these structures other changes have been taking place, as follows: First, owing to the inequalities in growth, there has been a considerable ventral shifting of the two branchial regions, accompanied by a marked dorso-ventral flattening of the pharyngeal cavity, so that the extent of its strictly lateral walls is greatly reduced. Thus instead of being situated on the sides of the pharynx the gill arches soon come virtually to occupy its floor, upon either side of a median strip which is relatively wide anteriorly and narrow posteriorly. Hence the new gills do not project laterally, but tend to hang

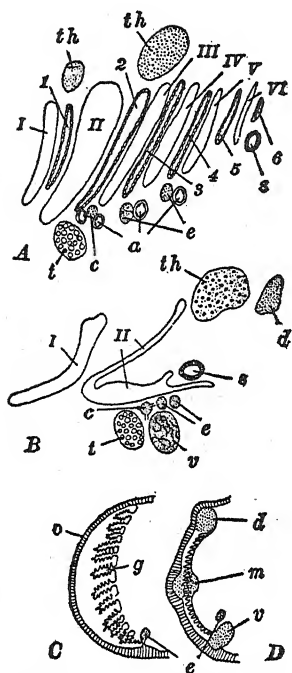


Fig. 105. — Diagrams of derivatives of visceral pouches and arches in Frog. From Kellicott (*Chordate Development*). After Maurer, with Greil's modification. A. Lateral view, Frog larva. B. Lateral view, after metamorphosis. C. Transverse section through gill of Frog larva. D. Transverse section through gill region just after metamorphosis; gills still visible.

a. Afferent branchial arteries. c. Carotid gland. d. Dorsal gill remainder. e. Epithelioid bodies. g. Internal gills. m. Middle gill remainder. o. Operculum. s. Suprapericardial body. t. Thyroid body. th. Thymus bodies. v. Ventral gill remainder. I-IV. Visceral arches. I. Mandibular arch. II. Hyoid arch. III-VI. 1st to 4th branchial arches. 1-6. Visceral pouches. (1. Hyomandibular pouch. 2-6. 1st to 5th branchial pouches).

downward into the opercular chamber (Fig. 104). Furthermore, the direction of the arches is not at right angles to the long axis of the pharyngeal floor. Instead they run diagonally backwards and outwards from the somewhat triangularly shaped median strip to the sides. From the borders of this strip which run almost at right angles to the gill arches, flaps of tissue now grow postero-laterally so as to cover these arches at their inner and more anterior ends. The two flaps, moreover, become continuous with one another at their posterior and median extremities, so that actually only a single V shaped flap exists, whose posteriorly directed apex is attached to, and overlaps, the narrowest region of the median strip. At the same time on each side a somewhat lesser flap develops from the lateral and dorsal wall of the pharynx along a diagonal line parallel with, but slightly posterior to, the respective side of the flap arising from the floor. These dorso-lateral flaps then grow anteriorly, medially and slightly downward, and because of the present close approximation of the pharyngeal floor and roof, they almost meet the lateral portions of the outgrowth from the former. The single ventral, and two dorso-lateral flaps, thus indicated are termed *velar plates*, and their arrangement is obviously such that only a narrow slit on either side leads from the pharynx to the gill chamber. It is these plates, together with toothlike processes on the inner sides of the gill arches, called *gill rakers*, which tend to prevent the escape of food, while allowing the free passage of water. Finally at the time of metamorphosis the gill pouches and the gill cavity are filled by

proliferated cells, while the mass thus formed is later absorbed leaving the gill slits closed.

Structures Derived from Vestiges of the Gill Pouches.—Just before hatching, proliferations of cells occur from the dorsal ends of the hyomandibular and first branchial pouches. Those from the hyomandibular pouch presently disappear, but those from each of the first branchial pouches form a cell mass. These separate from the pouches (about 12 mm.), and eventually take up their position back of the auditory capsules near the surface of the head. They are the *thymus bodies* (Figs. 105, 106).

From the ventral ends of the first pair of branchial pouches there occurs, at about the 9–10 mm. stage, a proliferation of cells. These cells, together with the anastomosis of the proximal ends of the afferent and efferent blood vessels of the first branchial arch (see below) form the so called *carotid glands*. Though long usage has apparently firmly fixed the title of gland upon these structures, they are not glandular in histological appearance or in function. They consist rather of a spongy network which performs an important service in helping to secure a relatively aerated blood supply for the internal carotid artery of the adult Frog. While the ventral ends of the first branchial pouches thus help to form the carotid glands, cells from the ventral ends of the second and third branchial pouches give rise to what are known as the *epithelioid bodies*.

The fifth pair of branchial pouches never actually develop as such but become mere masses of tissue known as the *ultimobranchial bodies* (*suprapericardial*).

The Thyroid.—This organ appears before hatching as a median longitudinal evagination from the floor of the pharynx in the form of a solid rod. Later (about 10 mm.), this separates entirely from the phar-

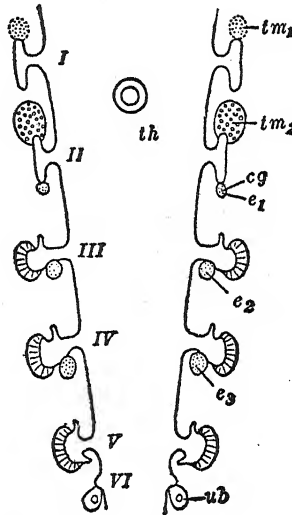


Fig. 106.—Diagram of the branchial pouch derivatives in the Frog. From Kellicott (*Chordate Development*). After Maurer, with Greil's modification.

cg. Carotid gland. e₁, e₂, e₃. Epithelioid bodies. th. Thyroid body. tm₁, tm₂, Thymus bodies. ub. Ultimobranchial body. I–VI. First to sixth visceral pouches (I. Hyomandibular II–VI. First to fifth branchial pouches).

206 THE FROG: LATER OR LARVAL DEVELOPMENT

ynx, and divides into two lateral parts which eventually become vascular.

The Lungs. — They appear just after hatching as a pair of solid posteriorly directed proliferations from the ventral side of the pharynx just back of the rudiment of the heart. The pharynx at this point is later depressed, and partially constricted off from the part above it as the *larynx*. The opening left between the pharynx and larynx is the *glottis* (Fig. 90). The lungs soon acquire cavities, and as they grow, become spongy and vascular. Part of their tissue is derived from the splanchnic mesoderm, only the inner lining being endodermal.

In connection with the origin of these organs it may be noted that there have been two general theories concerning their phylogenetic history. One school has regarded the lungs as coming from a modified swim bladder, while the other has considered them as developments of what were once a seventh pair of gill pouches. The latter notion at least has the merit of preserving a continuity of function in the forerunner of the respiratory organs of air breathing Vertebrates.

Further Development of Liver. — The liver rudiment has already been noted as a small endodermal diverticulum extending back slightly, beneath the yolk mass. The anterior wall of this diverticulum becomes folded and thickened, partly by the addition of scattered mesoderm and yolk cells (Fig. 90). This is the liver proper, the posterior part of the original outgrowth becoming partially constricted away from it as the *gall bladder*. The original connection with the fore-gut remains as the *bile duct*. These organs become well developed during the larval stage.

The Pancreas. — At the posterior margin of the opening of the bile duct into the fore-gut, a pair of outgrowths arise connected with the gut by a single piece of tissue, the future *pancreatic duct*. The free ends of these outgrowths then grow forward and fuse in front of the bile duct. Later they are joined by a mass of tissue which originated from the dorsal wall of the gut, and the three elements thus fused constitute the *pancreas*. Eventually the pancreatic duct comes to open into the bile duct very near to the point where the latter joins the gut, instead of directly into the gut itself.

With respect to the histogenesis of this organ, it appears that the islets of Langerhans in many species of the Frog at least, arise first from the endodermal cells of the primitive pancreatic anlage. Later these are added to by cells from the ductules. During metamorphosis some of the acinous cells degenerate, while the remainder persist as the

cells of the pancreatic tubules. The islet cells, on the other hand, become more aggregated, and develop two characteristic types with respect to staining capacity (Janes, '38).

The Esophagus and Stomach. — Shortly subsequent to hatching, the portion of the fore-gut between the future glottis and the opening of the bile duct elongates, and the anterior part of it becomes the *esophagus*. For a brief time the aperture between the latter and the pharynx is closed, but reappears at about the time the mouth opens. The posterior part of the above fore-gut region dilates slightly and assumes a transverse position as the *stomach*. This organ remains inconspicuous, however, until the time of metamorphosis, when it enlarges somewhat.

THE MID-GUT

The mid-gut is that portion of the archenteron lying above the large yolk mass at the time of hatching. After hatching, the yolk, and some of the cells of its floor are rapidly absorbed, and it begins to elongate. The front portion extends across the body in the form of a loop, the *duodenum*, which with the remainder is soon thrown into a double spiral. The coils of this spiral have a total length about nine times that of the body, but this is shortened about one third during metamorphosis.

THE HIND-GUT

The Rectum. — This terminal part of the gut originates with a relatively slight amount of growth from the small portion of the archenteron remaining between the yolk mass and the posterior body wall. It will be remembered that the endoderm of this region had come into contact with the ectoderm which had become invaginated to form the proctodaeum. About a week before hatching a perforation occurs at the point of contact forming the *anus*, while the rectum itself becomes slightly dilated. In this connection it is of interest to note that the proctodaeal portion of the blastopore which in the Frog closes with the rest of this orifice, and later reopens, in the Salamander always remains open. Thus the temporary closure in the former animal is probably a secondary or non-primitive characteristic.

The Postanal Gut. — As the tail region develops, the notochord and nerve cord extend into it, but since the proctodaeal region does not move backward, the neurenteric canal is drawn out into a small tube beneath the posterior end of the notochord. Somewhat before hatching it breaks away from the neural tube and persists for a brief period as the *postanal gut*.

The Cloaca and Urinary Bladder.—The general region where the endoderm of the rectum joins the ectoderm of the proctodaeum constitutes a chamber called the *cloaca*. It has been said that the cloaca is in fact all ectodermal and therefore proctodaeal, but this seems to the writer highly doubtful and extremely difficult, if not impossible, to prove. The reason for this doubt is that the pigment which at first marks the ectodermal cells, later becomes rather diffused, and the exact boundary of the original fusion of rectum and proctodaeum is obliterated. At all events the point at which the rectum may be judged to end, i.e., to open into the cloaca, is technically the *anus*. The dorsal walls of the cloacal chamber also receive the *urinogenital ducts*. Finally at metamorphosis the ventral part of the cloaca gives rise to an anteriorly directed outgrowth within the body cavity; this becomes the *urinary bladder*. In the higher animals this bladder is endodermal, and although as indicated above it is impossible to be certain, it seems highly probable that it is so here. One difference between Amphibians and some of the higher forms which is evident, however, is the fact that in the Frog and its relatives, as noted, the above ducts do not open into this bladder, but into the dorsal wall of the cloaca.

INTERNAL DEVELOPMENT: THE FURTHER DEVELOPMENT OF THE NOTOCHORD AND MESODERM

THE NOTOCHORD

When last indicated the notochord was merely a rod of undifferentiated cells with a considerable curvature at its anterior end to conform to the cranial flexure of the brain. By the 4 mm. stage, however, the cells of this rod have become vacuolated, intercellular vacuoles have also appeared, and the anterior curvature so far as the rod is concerned has almost vanished (Fig. 89). At the same time around the notochord there presently develop two sheaths. The outermost, known as the *primary* or *elastic sheath*, is formed from the most superficial chorda cells. The *secondary* or *fibrous sheath* lies within the latter and is formed of the chorda epithelium.

THE SOMITES

When last considered, the segmental plates had divided into four pairs of somites. This process continues posteriorly until there are thirteen such pairs, extending from just back of the auditory capsules to the

base of the tail. Within the latter organ the number is much larger and somewhat variable. Thus in a 5.5 mm. larva there may be all told as many as forty-five. Sometime after hatching, however, the first two pairs disappear, and those in the tail are of course all lost during metamorphosis; there thus remain eleven well-defined somites in the body region. Meanwhile, as these somites are formed they have been undergoing certain changes, as follows:

Each somite it will be recalled consists of an outer layer of cells called the cutis plate, and an inner larger mass, the myotome. From the inner and ventral edges of the myotome (about 5 mm.), loose *sclerotomal* cells are proliferated (Fig. 86). These cells then migrate medially and dorsally between the rows of myotomes on the one hand, and the notochord and nerve cord on the other. Eventually they thus form a layer about the latter structures known as the *skeletogenous sheath*. This ultimately (see below) gives rise to the cartilage and finally the bone which forms the centra of the vertebrae together with their transverse processes and neural arches. There are nine vertebrae thus formed in such a way that they alternate with the myotomal elements of the somites. The skeletogenous elements of the last two of the eleven somites have a somewhat different history, as will be indicated later.

At about the same time that the sclerotomal tissue is being proliferated, there are developing, within the myotomes, *muscle fibrillae*, which are to form the muscles of the back. Also from the outer ventral edges of the myotomes and from the ventral edges of the cutis plates or dermatomes, outgrowths extend down next to the ectodermal wall. These are to form the ventral body musculature, and in the region of the limbs, their musculature as well. The main part of each cutis plate breaks up and some of the cells from these plates form the dermal layer of the dorsal region, while others migrate between the myotomes to form connective tissue. It would appear that the dermis of the ventral regions is not derived from the dermatomes at all, but from part of the somatopleure, as has been demonstrated for the Chick (see below). Partial confirmation for this view has been furnished for the Amphibia by the experiments of Detwiler ('37) already cited. He has shown that although absence of somites (including the dermatome) prevents spinal ganglion formation, the dermis of the operated side is present as usual. It might also be noted here that virtually all, if not all, pigment in the Amphibia is ectodermal in origin, that of the later stages coming mainly from the neural crests. This is true not only for pigment in the epidermis, but for that in the dermis and viscera as well (DuShane, '38).

210 THE FROG: LATER OR LARVAL DEVELOPMENT

Finally, as indicated above, the mesoderm in the region where the segmental plate separates from the lateral plate constitutes the nephrotome, and is concerned with the formation of the excretory system. This will be described later.

THE GENERAL COELOM

The beginning of the coelomic spaces in the two lateral plates has already been described. These spaces continue to extend downward, until in a short time they meet one another beneath the gut and fuse. Thus in the trunk region, the coelom or *splanchnocoel* becomes continuous ventrally from one side of the embryo to the other.

Dorsally, the lateral plates of mesoderm on each side press up and in, between the dorsal wall of the gut and the notochord, until they meet. The splits in these plates then follow, but never quite reach each other, and hence the splanchnocoel never becomes continuous dorsally; there is always a thin but double-walled sheet of cells separating the right and left cavities. This is the *dorsal mesentery*. The gut as it develops is therefore slung from the dorsal wall by this mesentery, and completely encased in the splanchnic mesoderm.

INTERNAL DEVELOPMENT: THE CIRCULATORY SYSTEM

THE HEART AND PERICARDIAL CAVITY

The Primitive Cardiac Tube. — It will be recalled that when last mentioned the heart consisted merely of a few scattered endothelial cells lying between the endodermal floor of the pharynx and the mesoderm. It will also be remembered that upon either side of the mid-line this mesoderm had developed within itself a space which was designated as a rudiment of the pericardial cavity (Fig. 85, *C*). These spaces now enlarge, and the mesoderm forming their uppermost walls presses up and around each side of the above mentioned endothelial cells so as to separate them from the overlying pharynx. Meantime these cells have become arranged in the form of two parallel tubes (Fig. 107, *A*), which very shortly become more or less completely fused into a single tube (Fig. 107, *B*) extending throughout the region. Presently the in-pushing mesoderm from either side meets and fuses above this tube, so as entirely to surround it (3-6 mm.), (Fig. 107, *B, C*). The latter with its covering now represents the complete rudiment of the *heart*. The endothelial portion, as noted, forms its lining, the *endocardium*, while the

mesodermal envelope gives rise to the muscular wall, or *myocardium*, and the close fitting covering of the latter, the visceral pericardium.

From the method of its formation, it is evident that this tubular heart will at first be attached to the walls of its pericardial cavity by both a dorsal and ventral sheet of mesodermal epithelium, or *mesocardium*. The dorsal sheet was formed like that which suspends the gut, by the fu-

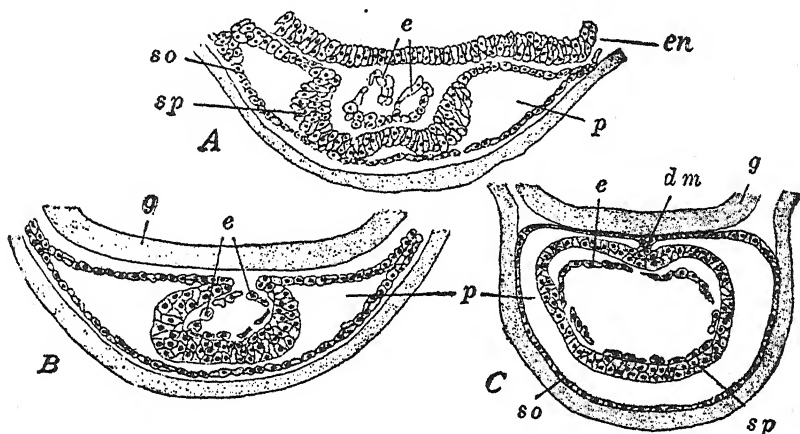


Fig. 107. — Sections showing the formation of the heart in the Frog. From Kelliecott (*Chordate Development*). A. Section through pharyngeal region of *R. temporaria*. After Brachet. B, C. Sections through the same region in older embryos of the smaller Frog, *R. sylvatica*. A. 3.2 mm. embryo. Endothelial cells becoming arranged in the form of a double tube. B. Embryo of about 3 mm. C. Embryo of 5-6 mm. The single heart tube established; dorsal mesocardium still present.

dm. Dorsal mesocardium. e. Cardiac endothelial cells. en. Endoderm. g. Wall of gut (pharynx). p. Pericardial cavity. so. Somatic layer of mesoderm (future parietal wall of pericardial cavity). sp. Splanchnic layer of mesoderm (future myocardium plus visceral wall of pericardial cavity).

sion of the sheets of mesoderm pushing in from each side. The ventral sheet, on the other hand, has existed from the start as the median strip separating the two pericardial rudiments. Thus the pericardial space remains temporarily divided along this middle line. Meantime, as indicated above, the lateral coelomic spaces in the trunk region have extended ventrally, and now each side of the pericardial cavity communicates posteriorly with these spaces. The next step involves the entire disappearance of the ventral mesocardium, followed very soon by the disappearance of the dorsal mesocardium also, except at its anterior and posterior ends.

At this point it is worth pointing out that all Vertebrate hearts develop in essentially the same manner, except for some of the later de-

212 THE FROG: LATER OR LARVAL DEVELOPMENT

tails involving the development of septa and orifices. That is, they all start with a pair of straight tubes which shortly fuse into one, as has been described, and this tube then develops in the manner about to be indicated to arrive at the adult condition. Since this is true it would be

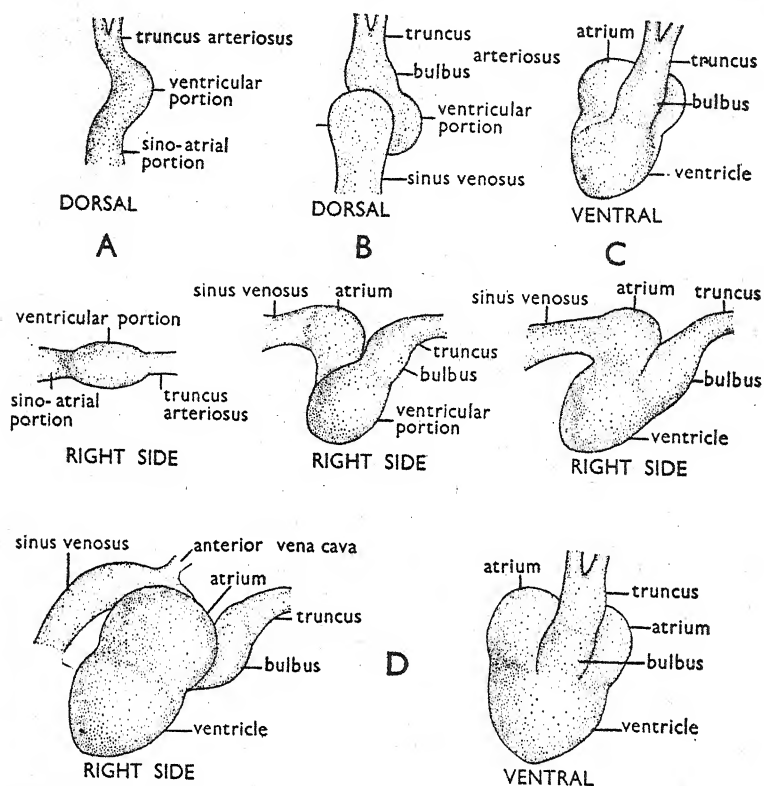


Fig. 108. — Stages in the development of a Vertebrate heart. These figures are primarily of the Frog heart, but would apply almost equally well to that of the Chick or Mammal (see text). The earliest stage is *A*, and that of an essentially adult heart is *D*. There are two views of each stage as indicated on the figure.

well for the student to follow the ensuing description carefully, and be sure that it is clearly understood.

As the already-mentioned mesocardia disappear, the tubular heart begins to increase in length, and by so doing becomes twisted in the following manner. The straight tube first develops a marked bend to the right (Fig. 108, *A*). The broad apex of the bend then moves ventrally, posteriorly and slightly to the left. Upon completion of this movement

we find that what amounts to a loop has been thrown into the originally straight tube (Fig. 108, *B*). The posterior limb of this loop extends ventrally and then curves outward to the right to form the wide apex. From the latter the ascending limb proceeds dorsally, slightly anteriorly and leftward into the median plane. Thus the two ends of the loop, anterior and posterior, are still in essentially the same straight line. Anteriorly the ascending limb of the tube divides at its upper extremity into certain vessels which pass dorsally into the visceral arches. These will be described presently. At the posterior end, on the other hand, the tube comes into immediate and close contact with the anterior surface of the yolk mass which is in process of developing into the liver (Fig. 90, *A*). In connection with the latter certain vessels are forming which will also be discussed more fully below.

It is now possible to indicate how the parts of this twisted tube give rise to the adult structures for which they are destined. As will immediately become apparent, not all of them belong to the heart proper. Nevertheless, because of their very close connection and simultaneous development it is convenient to describe them together.

Sinus Venosus Vitelline Veins and Atria. — Beginning at the posterior end it has just been noted that the heart tube abuts against the developing liver. Forming on the antero-ventral surface of the latter organ are two vessels, the *vitelline veins*, which become continuous antero-dorsally with the posterior end of the heart tube. The fused region of their entrance to the tube later becomes dilated to form the *sinus venosus*, while just anterior to this another enlargement occurs. This latter enlargement is the atrial portion of the heart proper, and presently there grows down from its roof a sheet of tissue dividing it into right and left chambers. These chambers are the *atria* of the Frog heart, and the sheet of tissue is the *inter-atrial septum*. It is further to be

* There has been considerable confusion over the definition of the terms *auricle* and *atrium*. According to the virtually universal usage of American medical men in human anatomy the two upper chambers of the heart are "atria" which have earlike appendages or "auricles" attached to them. In many of the lower animals including the Frog, however, there are no such appendages, i.e., there are in the strict sense no auricles, only atria. It should be noted that among British medical men the term auricle is frequently more loosely used to include all of each upper chamber, though they do sometimes refer to the auricular appendages of the atria. Also among zoologists the terms *auricle* and *atrium* are used as essentially synonymous. Nevertheless, there is good historical and logical precedent for the strict definition of these terms adopted by American human anatomists. Hence, since many students of embryology are sure to be premedics, the present writer intends to try to save them future confusion by adherence to the more precise definition of atrium and auricle throughout this text.

214 THE FROG: LATER OR LARVAL DEVELOPMENT

noted in this connection that the growth of this septum occurs in such a manner that the sinus venosus comes to open into the right atrium. The left atrium, on the other hand, eventually receives the pulmonary veins (see below).

The Ventricles, Bulbus and Truncus Arteriosus.— While these events are taking place in the postero-dorsal extremity (atrial region) of the looped tube, the curved apex of this tube connecting the descending limbs is expanding. As it does so, it incorporates into itself the ventral part of the descending limb not involved in forming the atria. This expanded portion of the tube constitutes the *ventricle*. In the case of the Frog, of course, it contains no dividing septum. Its wall, nevertheless, becomes greatly thickened by the development of muscular tissue, some fibers of which traverse the ventricular chamber itself forming partial partitions. These, in connection with other factors, are said to help prevent the mixture of the two classes of blood received from the respective atria (Fig. 108, C).

Later, as a result of a rotation of the whole structure about an axis passing transversely between the atria and ventricle, the ventricle assumes its definitive posterior position. Finally the ascending limb of the original tube, also as a consequence of this rotation, comes to run more or less anteriorly from the ventricle across the ventral side of the atria. It is not strictly part of the heart, but constitutes a thick walled vessel with two enlargements in it. The one nearer the ventricle is the *bulbus*, and the more distal less prominent one the *truncus arteriosus* (Fig. 108, D). Within the latter extending throughout its length there eventually develops from the dorsal wall a somewhat spirally twisted flap which partially divides the truncus into two channels. It never fuses with the ventral wall of the truncus so that the division remains incomplete. This flap is known as the *spiral valve* and functions in the adult to help keep separate the aerated and unaerated blood coming from the ventricle.

With respect to the initiation of functioning of the parts of the heart tube the following may be said: pulsation in all Vertebrate hearts so far as known begins long before any innervation, it being the nature of this particular type of muscle to contract rhythmically. This rhythmical contraction which begins within the anterior two thirds of the heart tube, almost at once assumes the form of a peristaltic wave moving from the posterior point of initiation anteriorly. This point is shifted backward as the length of the tube increases, and as might be expected it is this region which sets the pace for the rate of beat. This has been

clearly demonstrated for *Amblystoma* by Copenhaver ('39, '45) by cutting the tube at various places and times so as to show the inherent rates of the separated parts. By such experiments he has made clear that the posterior part of the tube, i.e., the region where the pulsation ultimately starts has a faster inherent rate than more anterior parts. Not only is this true, but interchange of posterior parts between species having different heart rates causes the imposition of the rate of the transplanted posterior part upon the anterior part of the host heart with which it has fused. In view of these facts it is not surprising to find that in the completed heart the beat is initiated and its rate determined in the sinus, which arises from the posterior end of the original tube. However, in the adult organ the situation is altered to this extent: though the beat is always initiated in the sinus, its inherent rate is modified by nervous control to meet the demands of changing conditions.

Isolation of the Pericardial Cavity.—Most of the above processes take place in the development of the heart before or shortly after the tadpole hatches (7–12 mm.). One step which remains until considerably later, however, is the separation of the pericardial cavity from the general coelom which lies posterior to it. This is accomplished by the outgrowth of folds of *peritoneum* (epithelial lining of the coelom) from the lateral coelomic walls, in company with the ductus Cuvieri (see below). The partial transverse wall thus formed is then augmented medially by the splitting off of peritoneal tissue from the anterior face of the liver. The entire partition is not completed until metamorphosis, when it is known as the *septum transversum*.

DEVELOPMENT OF BLOOD VESSELS AND CORPUSCLES

The blood vessels develop out of the mesenchyme and the splanchnic mesoderm by a rearrangement and differentiation of the cells to form a flat endothelium which constitutes the inner lining of all the vessels. It is entirely similar to, and continuous with, the endothelial lining (endocardium) of the heart which has just been described (Figs. 89 and 107). The muscular and connective tissue coats are likewise differentiated from mesoderm and added later, the muscle being much more abundant in the arteries and the connective tissue in the veins. In connection with these processes it should be emphasized that the early endothelial tubes do not originate as such at some one place, e.g., the heart, and simply grow outward from there as immediately continuous structures. They rather appear as disconnected sections or vesicles which grow toward each other until they are united. However, though it is true that the ves-

sels do not originate at one point, the procedures indicated do occur first in the more proximal regions of the embryo, and particularly in the vicinity of the heart. It is important to bear these facts in mind whenever the development of blood vessels is referred to, not only in the Frog, but also in any other Vertebrate for the method of formation is the same in all.

The corpuscles are formed chiefly from patches of splanchnic mesoderm on the ventral side of the yolk mass, from whence they find their way into the developing vessels. These patches are called *blood islands*. It appears, however, that the corpuscles produced by the islands do not last long, but are replaced by corpuscles from other blood-forming centers, particularly the spleen under stimulation by the liver (Goss, '28; Cameron, '41; Copenhaver, '43). In Salamanders a diffusible substance from the endoderm seems to aid haemoglobin formation, at least in the island corpuscles (Finnegan, '53).

The Arterial System. — A few days before hatching (4–5 mm.), the *dorsal aorta* develops as stated, just above the gut, and in the pharyngeal region is divided into two *lateral dorsal* or *suprabranchial aortae*.

The Visceral Arch and Gill Circulation. — At about the same time the blood vessels of the visceral arches also develop in the following manner:

Vessels appear in the branchial arches and a little later the two anterior pairs become connected ventrally with the truncus arteriosus and dorsally with the corresponding suprabranchial aorta. Presently similar connections are also established by the other two pairs. Thus complete loops or *aortic arches* are formed in all but the mandibular and hyoid arches. Here no real aortic arches ever develop, though certain transitory vessels appear for a time.

As the external gills now begin to form, the following changes occur in the first, second, and third branchial arches: A second looped vessel appears external to the primary aortic (branchial) vessel, the new vessel being attached to the primary vessel dorsally and ventrally (Figs. 103, C; 109, B). This new loop now extends out into the tissue of the corresponding external gill, where the two sides of the loop are connected by capillaries. Thus, for the time being, it is possible for the blood to go through either the original primary loop or the new gill loop and its capillaries. The greater part of the blood, however, takes the latter course. Hence it passes out from the truncus arteriosus along the more ventral and external side of the gill loop, which is therefore *afferent*, and back along the dorsal side, which is therefore *efferent*.

When the external gills disappear, the ventral limb of the external loop (i.e., the section *ab*) remains to form the afferent vessel of the internal gills (Figs. 103; 109). The efferent vessel, with which it then becomes connected by capillaries, is the more ventral part of the original primary loop (section *x*). Meanwhile, this primary loop breaks its main ventral connection at the point where the external loop branched off from it. Thus during the remainder of larval life all the blood in the arches has to go through the internal gill capillaries. Since the fourth

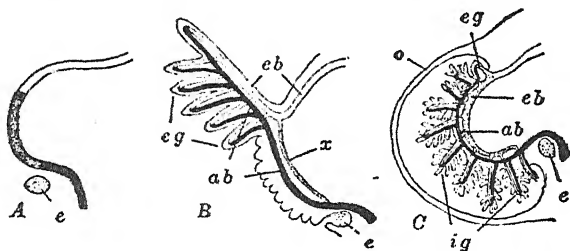


Fig. 109. — Diagrams of the second aortic arch of the adult Frog and tadpole. From Kellicott (*Chordate Development*). After Maurer. A. The continuous second (main systemic) aortic arch of the adult; showing the parts corresponding with the larval vessels. B. External gill and associated vessels in young tadpole. C. Internal gill and associated vessels in the tadpole after the disappearance of the external gills. *ab*. Afferent branchial artery. *e*. Epithelioid body. *eb*. Efferent branchial artery. *eg*. External gill. *ig*. Internal gill. *o*. Operculum. *x*. Direct connection between afferent and efferent branchial arteries, i.e., ventral part of primary loop.

arch never develops external gills, the vessels related to these particular structures never appear in it. Otherwise the history of the blood system within this arch is essentially similar to that just described in those anterior to it.

Changes in Gill Circulation at Metamorphosis. — The gills and their capillaries, including the major part of the afferent or external loops, gradually degenerate. At the same time the original primary loop vessels re-establish their ventral connections with the proximal parts of the afferent gill vessels. The primary vessels in the four pairs of branchial arches then undergo the following changes.⁴ The vessels of the first pair

⁴ It is to be noted in this connection that at least in some Frogs, as indicated in a preceding paragraph, no genuine aortic loops are formed in the mandibular and hyoid arches (Marshall and Bles on *R. temporaria*). In many other Vertebrates or their embryos, however (see the Chick), complete arteries do exist in these arches at one time or another, as well as in the four branchial arches. Thus in such cases the third aortic loop of the entire series is homologous with that in the first branchial arch referred to in the following account.

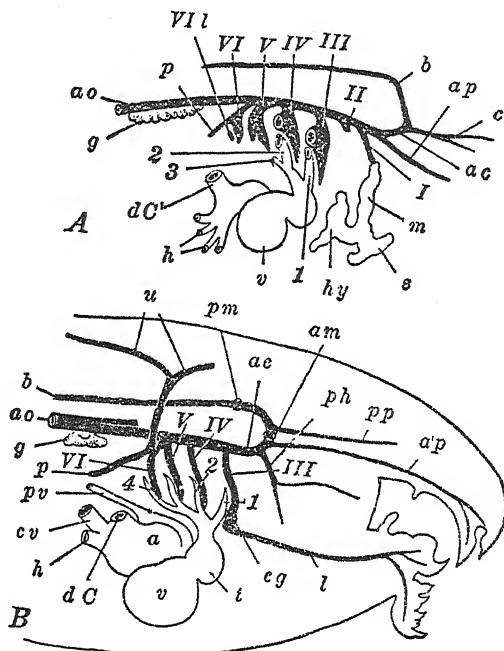


Fig. 110. — Diagrams of the branchial blood vessels in Frog larvae. From Kellicott (*Chordate Development*). After Marshall. (*Vertebrate Embryology*, courtesy of Putnam's Sons.) A. A 7 mm. larva (shortly after hatching). The vessels supplying the external gills are removed, only their roots being indicated. B. A 12 mm. tadpole. The vascular loops in the gills are omitted.

a. Atrium. ac. Anterior (internal) carotid artery. am. Anterior commissural artery. ao. Dorsal aorta. ap. Anterior palatine artery. b. Basilar artery. c. Anterior cerebral artery. cg. Carotid gland. cv. Posterior (inferior) vena cava. dC. Ductus Cuvieri. g. Pronephric glomus. h. Hepatic veins. hy. Hyoid vein. l. Lingual artery. m. Mandibular vein. p. Pulmonary artery. ph. Pharyngeal artery. pm. Origin of posterior commissural artery. pp. Posterior palatine artery. pv. Pulmonary vein. s. Vein of oral sucker. t. Truncus arteriosus. u. Cutaneous artery. v. Ventricle. 1-4. First to fourth afferent branchial arteries. I, II. Efferent arteries of the mandibular and hyoid arches. III-VI. First to fourth efferent branchial arteries. VII. Lacunar vessel of the fourth branchial arch.

of branchial arches retain their dorsal connections with the respective dorsal aortae, and with them form the proximal ends of the *internal carotids* which run forward into the head (Fig. 110). The vessels of the same arches are joined at their ventral ends by the *external carotids* or *lingual arteries* which have grown back from the floor of the mouth. Almost at the junction of the external and internal carotids on each side, the latter develops an enlargement consisting of spongy tissue. This is the carotid gland already referred to. It arises from a slight anastomosis between the proximal ends of the afferent and efferent aortic vessels of the first branchial arch, with the addition of some epithelial cells from the ventral end of the first branchial pouch.

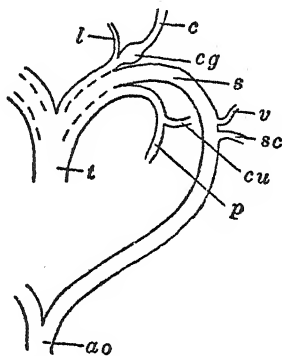


Fig. 111.—Diagram of the aortic arches and their chief branches in an adult Frog. From Kellicott (*Chordate Development*). Ventral view.

ao. Dorsal aorta. c. Carotid artery. cg. Carotid gland. cu. Cutaneous artery. l. Lingual artery. p. Pulmonary artery. s. Systemic arch. sc. Subclavian artery. t. Truncus arteriosus. v. Vertebral artery.

The vessels of the second pair of branchial arches also retain their dorsal connections with the lateral dorsal aortae, while the latter disappear anteriorly between this point and the first branchial arches (disappearance not shown in Fig. 110). Thus the vessels of the second branchial arches become the *main systemic arteries*. The vessels of the third branchial arches disappear. The vessels of the fourth branchial arches, having already given off branches to the lungs and skin, become the *pulmocutaneous arteries*. The portion of each of these vessels connecting it with the respective lateral aorta disappears after metamorphosis. Thus all the blood going to these aortic arches must henceforth pass to the lungs or skin.

It may be noted that in most of the air-breathing Vertebrates not all of the section of the fourth arch between the origin of the pulmo-cutaneous artery and the dorsal aorta, known as the *ductus Botalli*, completely disappears. Instead it remains as a vestigial strand. Among the Amphibians this is true of many of the Urodeles, but not of the Anura.

In conclusion the functions of certain of the rather special structures of the Frog heart whose development has been described may be briefly indicated. It will be recalled that muscle fibers in the undivided ventricle tend to act as partial partitions and to keep the kinds of blood in it

220 THE FROG: LATER OR LARVAL DEVELOPMENT

separated. The spiral valve in the truncus arteriosus then assists in guiding these different kinds to the proper pairs of arches. Thus the relatively unaerated blood leaves the heart first, and goes into the fourth arch on the way to the lungs and skin. Then the mixed blood is guided into the main systemic arches and external carotid. Lastly, the relatively aerated blood is forced through the carotid "gland" and into the internal carotid to the upper part of the head and brain (Fig. 111).

Other Arteries. — The *pharyngeal arteries* develop at about 9 mm. from outgrowths of the suprabranchial aortae, which at first connect with transitory vessels in the mandibular arches. At about the middle of each main systemic aortic arch a large branch is given off to the fore limb; it is the *subclavian*. The suprabranchial or lateral aortae come together to form the single dorsal aorta at about the level of the pronephros (see below). Throughout the remainder of its course this artery gives off several *lumbar arteries* to the body wall, as well as larger branches which supply the viscera (*mesenteric arteries*), and the hind limbs and adjacent regions (*iliac arteries*).

The Venous System.

The Hepatic and the Hepatic Portal Systems. — In discussing the development of the heart, it was noted that almost from the first two veins entered it posteriorly, i.e., the vitelline veins. Just at the point of entrance to the heart their fusion resulted in the formation of a common chamber, the sinus venosus. Between this point and the liver a further fusion of these veins occurs not long after hatching, and the result is for the time being the *hepatic vein* (Fig. 110). Although first mentioned in connection with the heart, the vitelline veins actually appear first on the ventro-lateral sides of the yolk mass, whence they pass along the sides of the yolk and liver to the heart. As noted, fusion early occurs anterior to the liver, but posterior to it the vitelline veins remain separate. The right vein within this region then disappears, and the left becomes the *hepatic portal vein*. It remains connected with the anterior hepatic vessel only through capillaries within the liver substance, while posteriorly it sends branches to the digestive tract. This vein with its branches and liver capillaries constitutes the *hepatic portal system*.

The Ducts of Cuvier, the Cardinal Veins and Their Derivatives. — Though the lateral body walls of the early embryo (before hatching) are separated somewhat from the sinus venosus, this structure remains connected with each wall by a broad mass of tissue. Within

each of these connections there presently develops a sinuslike vein, the *ductus Cuvieri*. These veins do not run horizontally from the sinus venosus to the body walls, but obliquely upward. At the points of union with the respective wall each ductus then gives rise to an anterior and a posterior branch within the wall itself. These are the *anterior* and *pos-*

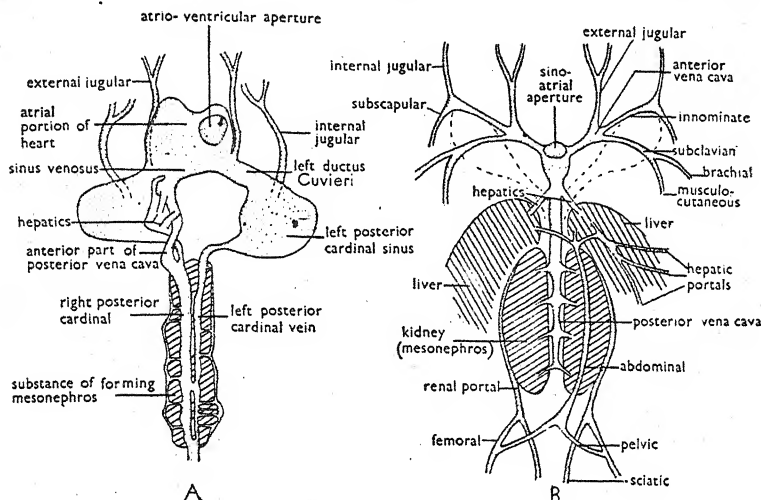


Fig. 112. — Figure *A* is a reconstruction in ventral view of the chief veins of a 10 mm. *Rana pipiens* larva made from serial cross sections, and enlarged 22.5 times. The ventricle of the heart is omitted, and the mesonephros is of course shown only diagrammatically to indicate its relative position. Figure *B* is a semi-diagrammatic representation in ventral view of the veins in an adult Frog which are derived from those shown in *A*, with the addition of the abdominal vein, as described in the text. The entire heart is omitted from this figure, and the dotted lines merely outline where the posterior cardinal sinuses would be if they were still present. It should be noted, as indicated by the labels, that the aperture in figure *A* is a completely different one from that represented in figure *B*. Also, it is to be emphasized that since figure *B* is near natural size, the two figures are on nowhere near the same scale. As usual, relative degrees of growth of parts account for many of the differences, especially in connection with the development of the anterior vena cavae.

terior cardinals. Presently there grows anteriorly from the base of each ductus Cuvieri a vein which extends into the floor of the mouth, the *inferior (external) jugular*. This situation is clearly in evidence at 10 mm. or earlier (Fig. 112, *A*). Later at about the point of origin of each inferior jugular there also grows toward the region of the respective future shoulder another vein which becomes the *subclavian*. At approximately the same time, so far as is known, the base of each ductus Cuvieri becomes extended somewhat, thus separating the place of origin of the respective inferior jugular and subclavian from the sinus venosus. The

222 THE FROG: LATER OR LARVAL DEVELOPMENT

short new section of vessel thus added to the proximal end of each ductus is then known as an *anterior vena cava*. The remaining portion of each ductus between the origin of the respective inferior jugular and the origin of the respective anterior cardinal, the posterior cardinals having meanwhile disappeared (see below), is henceforth called an *innominate*. Thus each anterior cardinal itself now becomes a *superior (internal) jugular*. At about the junction of each innominate vein and the respective superior jugular a backward curving vessel arises which is a *subscapular* (Fig. 112, B).⁵

Turning now to the posterior veins, each posterior cardinal will be found proceeding from the junction of the ductus Cuvier and anterior cardinal (superior jugular) backward through the pronephric region. Here it has the form of a broad sinus which more or less surrounds the pronephric tubules (see below). Posterior to this region, it turns sharply toward the median line and continues along the median side of the respective pronephric (Wolfian) duct to the cloaca (Fig. 112, A). Along its course, each of the cardinals receives branches from the body wall, and at their posterior extremities the two veins unite and receive the *caudal vein* which brings the blood from the tail.

At about the 10 mm. stage in *Rana pipiens*, modifications in this arrangement begin as follows: Along the median dorsal surface of the liver a new vein forms which empties into the hepatic vein anteriorly, and posteriorly unites with the right posterior cardinal just caudal to the pronephros (Fig. 112, A). At approximately the same time, or slightly later the posterior fusion of the posterior cardinals proceeds anteriorly in an intermittent manner into the region of the developing mesonephroi, and eventually it occurs throughout the extent of those organs. Thus is produced a *median cardinal vein* which, due to the manner of its formation, is continuous anteriorly with the new vein connecting the right cardinal with the hepatic. With the disappearance of the pronephros, the right cardinal, anterior to the point where the new vein has joined it, and all of the left cardinal, also disappear. The single median vein which results is called the *posterior vena cava*. It is to be noted that its posterior portion is really simply the former median cardinal vein, while its extreme anterior part is merely the old hepatic vein which receives branches from the liver. As the latter vein thus becomes part of the posterior vena cava opening into the sinus venosus,

⁵ There is no very complete description of just how some of the branches just indicated as arising subsequent to 10 mm. actually develop, though the early larval and the adult conditions are of course well known.

the branches which it receives from the liver substance become the *permanent hepatic veins* (Fig. 112, A).

Meanwhile it is to be noted that as the posterior cardinals fuse and the mesonephroi develop, there arises along the lateral border of each of these organs a new vein. Each of these veins then becomes connected with the median vein (posterior vena cava) by numerous channels through the mesonephric substance (Fig. 112, A). Indeed according to some accounts (Shore, '01) the cardinals simply fuse, and then are partially divided by the mesonephroi into three main parts, a median and two lateral, the undivided remnants constituting the connecting channels (Figs. 112, A; 113, A, B). Though this is Shore's description of the process, it seems to the writer that three fairly separate channels exist before the mesonephros has developed to any extent. The mesonephric (pronephric) ducts are of course present, however, and it appears that they may help to split off a lateral channel from each of the fusing, more medially placed, cardinals. It also appears to the present author that in many, if not in all, cases at the 10 mm. stage the undifferentiated mesonephric primordium (nephrotomal tissue) extends across the

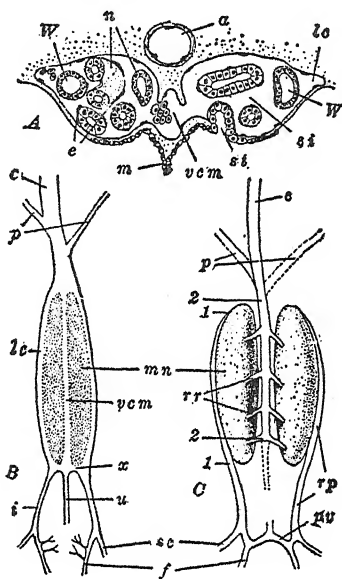


Fig. 113. — The development of the posterior part of the venous system in the Frog. From Kellicott (*Chordate Development*). After Shore. A. Portion of a transverse section through the posterior mesonephric region of an 18 mm. tadpole. B. Diagram of the veins of a 25-30 mm. tadpole. C. Diagram of the veins of the adult Frog.

a. Dorsal aorta. c. Vena cava. e. Nuclei of the endothelial lining of the mesonephric sinus, continuous with the vascular endothelium. f. Femoral vein. i. Iliac vein. lc. Lateral mesonephric channel of the posterior cardinal vein. m. Mesentery. mn. Mesonephros. n. Mesonephric tubules. p. Posterior cardinal veins (in C showing their original location). pv. Pelvic vein. rp. Renal-portal vein. rr. Revent renal veins. sc. Sciatic vein. st. Nephrostome. u. Caudal vein. vcm. Median mesonephric channel of the posterior cardinal vein. W. Wolfian duct. x. Connection between caudal vein and the lateral mesonephric channels. 1-1. Part of the renal-portal vein formed from the lateral channel of the posterior cardinal. 2-2. The posterior part of the vena cava formed from the median channel of the posterior cardinal vein.

224 THE FROG: LATER OR LARVAL DEVELOPMENT

median line in many places as a single mass just above the fusing cardinals. This mass then seems actually to be divided by the dorsally pushing median cardinal vein instead of the reverse process as usually described. Perhaps the real procedure is one of mutual interpenetration of mesonephric substance and veins as suggested in Figure

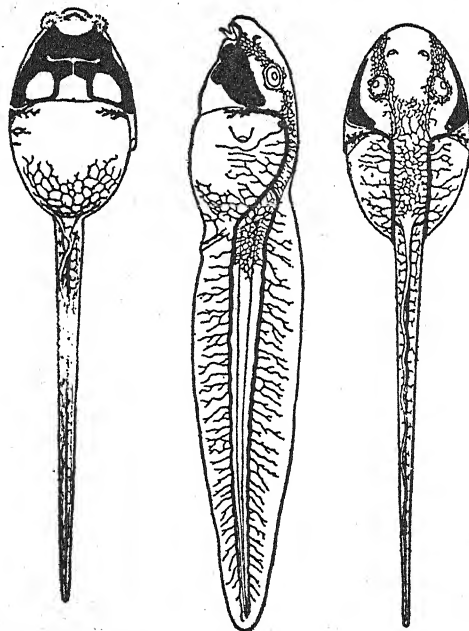


Fig. 114. — Ventral, lateral and dorsal views of the lymphatics in a 26 mm. tadpole of *R. temporaria*. From Hoyer. For description see text.

112, *A*. The writer regards this as most probable on the basis of his own observations. Be this as it may the ultimate result is that the lateral vessels develop to become the *renal portal veins*, and the channels connecting them with the median posterior vena cava are then the *renal veins*. Later with the appearance of the legs each renal portal vein is joined by an *iliac vein* which, as these appendages develop, divides at its distal end into the *femoral* and *sciatic veins*. Finally with the loss of the tail the part of the posterior vena cava caudal to the kidneys vanishes, so that most of

the blood from the posterior region of the body must pass through the renal portal vessels and the abdominal (see below) (Figs. 112; 113).

The Pulmonary Veins. — These begin to develop very early (6 mm.) as a dorsal offshoot from the sinus venosus. Later this offshoot opens into the left atrium, while at the lungs the single pulmonary vein divides so as to receive blood from each.

The Abdominal Vein. — At a relatively late period, a pair of veins from the sinus venosus extends back along the ventral body wall to the bladder, making lateral connections with the femoral veins. Just anterior to the bladder the two vessels then fuse; while still further forward the right one later disappears entirely. The remaining single vessel is the *abdominal vein*, which finally loses its connection with the sinus

THE PRONEPHROS AND SEGMENTAL DUCT 225

venous; it then acquires a connection with the hepatic portal vein, and also develops two branches opening into the capillaries of the liver (Fig. 112, B).

The Lymphatic System.— Just before hatching, the *anterior lymph hearts* appear to arise from a superficial plexus of veins between the third and fourth somites. They lie between the peritoneum and the integument, and soon become incased in muscle fibers. In connection with each "heart" there develop from other parts of the above venous plexuses two vessels just beneath the skin. One proceeds anteriorly, and the other posteriorly, while into these vessels drain numerous anastomosing capillaries; the latter eventually form the characteristic *subcutaneous lymph sacs* of the Frog. Sometime after hatching (26 mm.), the anterior vessels open downwards into large lymph sinuses in the branchial region (Fig. 114). The lateral posterior trunks unite at the root of the tail, and divide into a dorsal and a ventral vessel, which pass into it. The *thoracic ducts* seem to be outgrowths of the anterior lymph hearts, which extend posteriorly between the dorsal aorta and the posterior cardinal veins. When the hind legs appear, *posterior lymph hearts* develop from the segmental veins of that region also.

All the lymph hearts are guarded by valves between themselves and the lymph channels on the one hand, and between the hearts and blood vessels on the other. Thus the lymph always passes into the blood, never in the reverse direction.

The Spleen.— At about 10 mm. there appears in the mesentery, on the anterior mesenteric artery, just dorsal and posterior to the stomach, a collection of lymph cells. They multiply, and later (25 mm.) the cell mass becomes very vascular. The body thus formed is the *spleen*.

INTERNAL DEVELOPMENT: THE LARVAL EXCRETORY SYSTEM

Although both the larval and adult systems are paired, we shall refer only to the development upon one side. This is done with the understanding that the processes on the opposite side are identical.

THE PRONEPHROS OR HEAD KIDNEY, AND THE SEGMENTAL DUCT

The Pronephros.— When last described, the somatic wall of the nephrotomal region had thickened until it slightly overhung the side

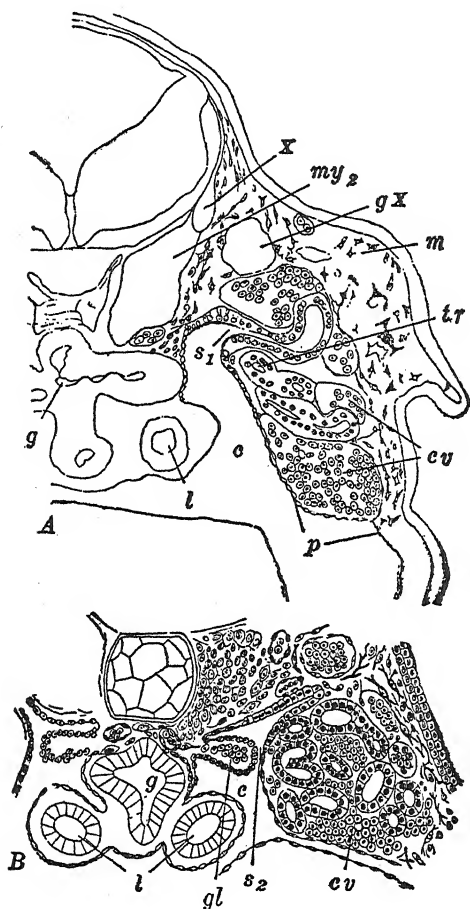


Fig. 115. — Sections through Frog larvae illustrating the later development of the pronephros. From Kellicott (*Chordate Development*). A. A section through the first nephrostome of a larva of *Rana sylvatica* of about 8 mm., with prominent external gills. After Field. B. A section through the region of the second nephrostome of a 12 mm. larva of *Rana temporaria*. After Fürbringer.

c. Coelom. cv. Sinuses of posterior cardinal vein. g. Gut cavity. gl. Glomus. gX. Ganglion nodosum (part of the ganglion of the vagus nerve). l. Lung. m. Mesenchyme. my₂. Second myotome. p. Peritoneum. s₁, s₂. First and second pronephric nephrostomes. tr. Common trunk. X. Root of vagus nerve.

of the lateral plate between it and the ectoderm; in the region of the second, third and fourth somites, cavities were beginning to appear within the thickening, especially in its lateral portion (Fig. 84). These laterally placed cavities now tend to run together so as to form in this region a continuous longitudinal lumen, the *common trunk*. At the same time, other spaces between this lumen and the coelomic cavity enlarge and unite with one another to form three separate tubules connecting the trunk with the coelom. These are the *pronephric tubules*, and each of them is opposite one of the three somites referred to. The opening of each tubule into the coelom is in the form of a funnel named the *nephrostome* (Fig. 115), which presently becomes lined with long cilia. The tubules, together with the common trunk, now become somewhat convoluted, and these convolutions begin to become imbedded in the sinus-like cardinal vein which partially surrounds them (Figs. 115,

116). At the same time the mass which is thus formed becomes enclosed on its dorsal and outer sides by connective tissue derived from the myotomes of this region and from the somatic mesoderm. This covering is termed the *pronephric capsule*.

Although not directly connected with the pronephric tubules, there develops with them another organ which because of its position and structure is probably concerned with their function. It arises as an out-pushing or fold of splanchnic mesoderm at the extreme dorsal limit of the coelom in the region just opposite the nephrostomes. In this way a

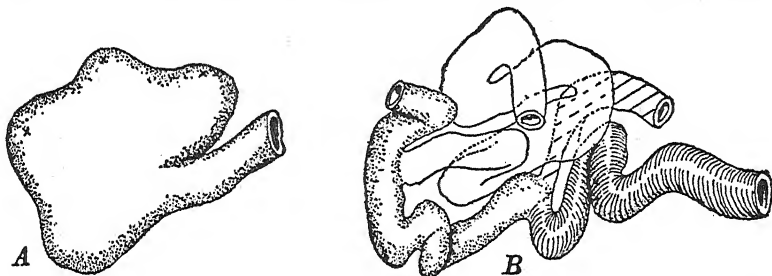


Fig. 116. — Total views of the pronephros of the Frog (*R. sylvatica*). From Kellcott (*Chordate Development*). After Field. A. Right pronephros of an embryo of about 3.5 mm. The crosses mark the location of the nephrostomes. B. Right pronephros of a larva of about 6 mm. First tubule dotted; second white; third obliquely ruled; pronephric (segmental) duct shaded with lines.

small mass of tissue becomes suspended directly opposite these openings. Presently numerous capillaries form within it and become connected with the nearby dorsal aorta. This vascular body is then called the *glomus*, and it has been shown by transplants in *Amblystoma* that the stimulus to its development depends upon the presence of the pronephric tubules (Fales, '35), even though the latter have no direct connection with it. The pronephric tubules, together with the *glomus*, may henceforth be referred to as the *pronephros* or *head kidney* (Figs. 116, 117).

The Segmental Duct. — So far as has yet been indicated, the larval kidney has no external outlet. While the above changes are going on, however, the lumen of the common trunk has extended backward through the lateral border of the nephrotome until it has established a connection with the cloaca. The outer portion of the nephrotome containing this lumen is then called the *pronephric* or *segmental* duct. Posterior to the fourth somite it gradually becomes more or less separated from the more median portion of the undifferentiated nephrotomal tissue which occurs in this region.

228 THE FROG: LATER OR LARVAL DEVELOPMENT

Changes Subsequent to Hatching. — This is approximately the condition reached at the time of hatching, when the tadpole is from 6–7 mm. long. The pronephros does not attain its maximum development, however, until the animal is about 12 mm. in length. During this particular

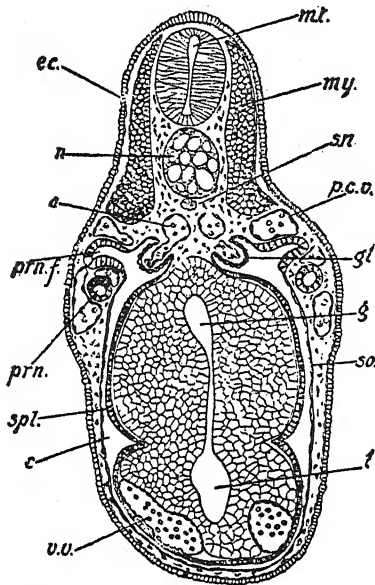


Fig. 117. — Transverse section of an advanced Frog embryo. From Jenkinson (*Vertebrate Embryology*).

mt. Medullary tube. *n.* Notochord. *sn.* Subnotochordal rod. *my.* Myotome. *a.* Aorta. *p.c.v.* Posterior cardinal vein. *prn.* Pronephric tubule. *prn.f.* Pronephric funnel (i.e., nephrostome). *gl.* Glomus. *c.* Coelom. *so.* Somatopleure. *spl.* Splanchnopleure. *g.* Gut. *l.* Liver. *v.u.* Vitelline vein. *ec.* Ectoderm.

period the pronephric tubules increase their convolutions to a considerable extent, and the coelomic space into which the nephrostomes open and in which the glomus is suspended becomes cut off ventrally from the main coelomic cavity. This is accomplished by the development of the lungs in this region (see Fig. 115). These organs are covered by a fold of the splanchnic mesoderm, and, as they grow, this covering fold is eventually brought into contact with the somatic mesoderm, with which it fuses for a short distance. The cavity thus formed, though it is separated from the coelom beneath, remains open to it both anteriorly and posteriorly. It is termed the *pronephric chamber*.

By the time the larva reaches a length of 20 mm., the head kidney begins to degenerate. Thus the pronephric region of the segmental duct becomes cut off from the part posterior to it. The former portion of the duct, together with the pronephric tubules and their nephrostomes, then gradually disappears;⁶

the glomus at the same time shrivels up, though remnants are visible even after metamorphosis. As the larval kidney is thus eliminated, its place is taken functionally by the mesonephros whose development is now to be described.

⁶ Hall states that during the degeneration of the pronephros the three nephrostomal openings, at least in *R. sylvatica*, always become fused into one, the common nephrostome (Fig. 118. C).

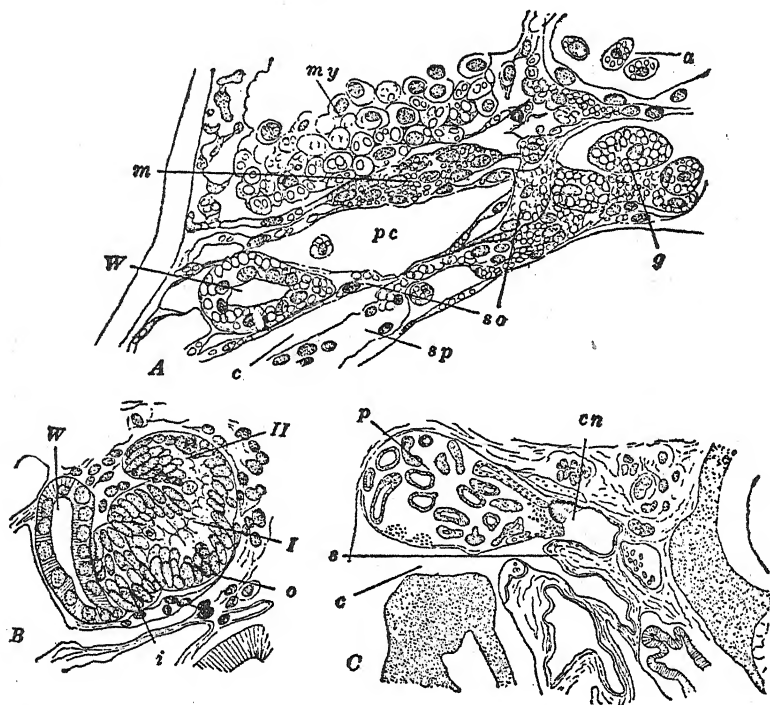


Fig. 118. — Sections through the developing mesonephros and the degenerating pronephros of *R. sylvatica*. From Kellicott (*Chordate Development*). After Hall. A. Section through the eighth somite of an 8.5 mm. larva. B. Section through the mesonephric rudiment of a 25 mm. larva. C. Section through the pronephric chamber and the common nephrostome of the pronephros of a 25 mm. larva.

a. Dorsal aorta. c. Coelom. cn. Common nephrostome. g. Germ cell. i. Inner tubule. m. Mesonephric rudiment. my. Myotome. o. Outer tubule. p. Remains of pronephros. pc. Posterior cardinal vein. s. Shelf cutting off the pronephric chamber from the remainder of the coelom. so. Somatic mesoderm. sp. Splanchnic mesoderm. W. Wolffian duct. I. Primary mesonephric unit. II. Secondary mesonephric unit.

THE MESONEPHRIC OR WOLFFIAN BODY

Posterior to the pronephros the outer margin of the nephrotome went to form the segmental duct. The inner portion medial to the duct appears meantime to have fused to some extent with that from the opposite side, thus forming a continuous mass ventral to the dorsal aorta, and above the fusing, or fused, posterior cardinal veins. This inner portion now starts to form the adult kidney in the following manner.

The Mesonephric Vesicle. — As indicated above, the inner part is for a brief time divided into segmental nephrotomes. These, however,

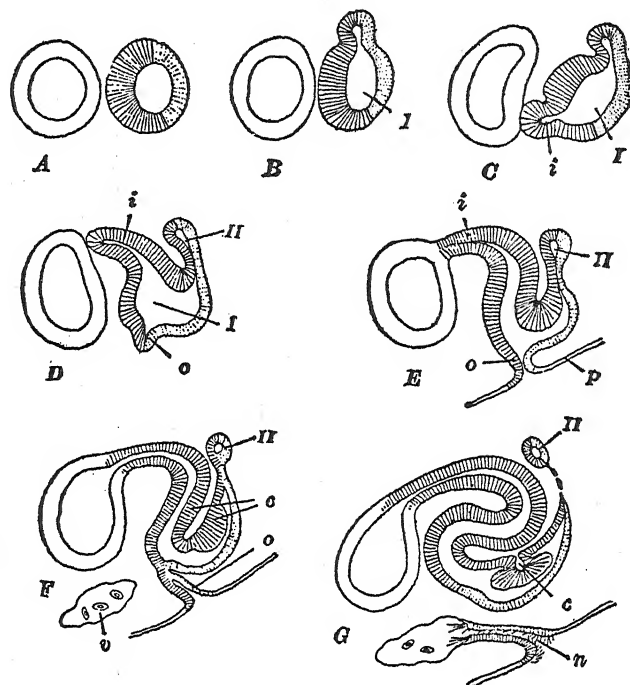


Fig. 119. — Series of diagrams illustrating the development of the primary mesonephric tubules in *R. sylvatica*. From Kellicott (*Chordate Development*). After Hall. The Wolffian duct is drawn in outline simply. The mesonephric vesicles are shaded; the somatic part of the tubule is shaded by continuous lines, the splanchnic part by dotted lines. A. Wolffian duct and simple mesonephric vesicle. B. Mesonephric vesicle dividing into the large primary mesonephric unit and the small dorsal chamber. The latter elongates antero-posteriorly and represents the rudiment of the secondary and later mesonephric units. C. Formation of the rudiment of the inner tubule. D. Inner tubule extending upward and toward the mesonephric duct; formation of rudiment of outer tubule. E. Outer tubule fused with peritoneum and rudiment of nephrostome thus established. Bowman's capsule forming. Commencement of differentiation of secondary mesonephric unit. F. Separation of nephrostomal rudiment from remainder of tubule. G. Connection of nephrostome with branch of posterior cardinal vein; separation of secondary unit.

c. Bowman's capsule. i. Inner tubule. n. Nephrostome. o. Outer tubule. p. Peritoneum. v. Branch of posterior cardinal vein. I. Primary mesonephric unit. II. Secondary mesonephric unit. Tertiary mesonephric unit not yet developed.

disappear almost at once so that a single nephrotomal band extends from the seventh to the twelfth somites. Within either side of this band there then arise a series of thickenings somewhat more numerous than the somites, and in each thickening there soon appears a cavity (Figs. 118, 119). This cavity, which is called the *mesonephric vesicle*, eventually becomes divided into two parts, the second and smaller part still later giving rise to a third. These parts are called *primary*, *secondary*, and *tertiary units*, in the order of their appearance, and their further development, though not simultaneous, is identical in character. It will be necessary, therefore, to describe the process in only one of the *primary units*.

The Development of a Primary Vesicular Unit.—Upon the dorsal side of the unit a small hollow outgrowth appears (Fig. 119, *B*). This, as later events prove, represents the rudiment of the secondary-unit, but for the present does not develop further. Next (Fig. 119, *C*), an evagination pushes out from the ventro-lateral side of the primary unit in the direction of the segmental duct. This is the *inner tubule*, which presently becomes connected with the segmental duct, the latter being henceforth known as the *mesonephric* or *Wolffian duct*. It is to be noted, moreover, that, by virtue of the partial rotation of the primary unit, this connection occurs dorsally rather than ventrally (Fig. 119, *D, E*). A part of the inner tubule later becomes greatly convoluted and the coils press down into the median cardinal vein (15 mm.), perhaps helping to divide the latter, as indicated above. Meanwhile there has grown out from what is now the ventral side of the unit, another evagination which presently become connected with the peritoneal (coelomic) cavity. This is the *outer tubule*, whose subsequent history in the Frog is very peculiar.⁷ It soon (20 mm.) breaks away from the main portion of the unit and acquires an opening into the lateral division of the median cardinal vein, i.e., the future renal portal vein. At the same time its opening into the coelomic cavity becomes ciliated as a typical *nephrostome*, this curious connection between body cavity and blood vessel persisting throughout life (Fig. 119, *F, G*).

The growth of these tubules has meanwhile been accompanied by a loss of the round or vesicular character of the region of the original primary unit. Thus between the point of origin of the secondary unit and that of the inner tubule, this region has become stretched out, and at the same time invaginated in a ventro-medial direction (Fig. 119,

⁷ Some authorities assert that the outer tubule probably never actually opens into the cavity of the primary unit from which it arises (Marshall Hall).

232 THE FROG: LATER OR LARVAL DEVELOPMENT

E, F, G). In this manner a cavity is produced which is later filled by a mass of capillaries connected with the dorsal aorta and also with the posterior vena cava. Such a capillary mass is called a *glomerulus*. The occurrence of the venous connection and the location of the structure within the kidney rather than in the coelom are two essential features in which a glomerulus differs from a glomus. The surrounding walls of the

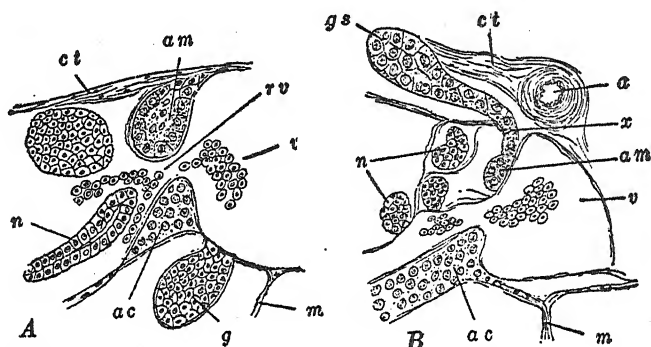


Fig. 120. — Parts of sections through young *R. temporaria*, showing the origin of the adrenal bodies. From Kellicott (*Chordate Development*). After Srdinko. A. Through 30 mm. tadpole. B. Through 11 mm. Frog after metamorphosis.

a. Dorsal aorta. ac. Corticle cells of adrenal body. am. Medullary cells of adrenal body. ct. Connective tissue. g. Gonad. gs. Sympathetic ganglion. m. Mesentery. n. Mesonephros. rv. Revehent renal vein. v. Vena cava. x. Point where ganglion cells enter mesonephros and adrenal body.

invaginated unit in which the glomerulus thus lies embedded then constitute *Bowman's capsule*, the capsule and capillaries together being termed a *renal corpuscle* or *Malpighian body*.

The occurrence of similar processes in the other units finally results in a mass of tubules, glomeruli, and nephrostomes, which constitute the adult *mesonephric organ* or *kidney*. This organ is virtually complete by the time metamorphosis is ended.

THE ADRENALS

Though in no sense a part of the excretory system, these organs always occur in such close connection with the kidneys that it seems best to describe them at this point. Indeed, in the mature Frog the relationship of the adrenals and kidneys is more intimate than in the higher Vertebrates, so much so that it is difficult to separate them. Thus in this animal, the former organs appear merely as an area of thin yellowish

tissue attached to the ventral side of the mesonephros. They are composed, however, of two kinds of cells, the so-called *medullary substance*, and the *cortical substance*, which originate as follows:

The cortical substance is so named from the fact that in higher forms it occurs on the outside of the organ, though this is not true of the Frog. Here it consists of anastomosing cells apparently derived (at about 12 mm.) from the mesonephric blastema cells (Segal, '53) near the cardinal veins. These cells form a meshwork into which branches from the veins soon penetrate. The medullary substance consists of pigmented cells which appear later. They are derived originally from sympathoblasts in the sympathetic ganglia of the mesonephric region, and become scattered throughout the cortical tissue (Fig. 120).

INTERNAL DEVELOPMENT: THE GENITAL SYSTEM

THE GONODUCTS

In the Male. — The *vas deferens* of the Frog is simply the mesonephric or Wolffian duct, which serves as both ureter and sperm duct. Posteriorly, in the region of the cloaca, each duct develops a glandular *seminal vesicle*. Anteriorly each *vas deferens* becomes connected with the respective testis as follows: From the latter certain strands of tissue known as rete cords (see below) develop into fine ducts which grow into each mesonephros along its median edge. Within the kidney these fine ducts become connected with the Bowman's capsules of some of the kidney tubules. The fine ducts together with the tubules of the kidney with which they thus connect then constitute the *vasa efferentia*, opening into each mesonephric duct (*vas deferens*).

At about 20 mm., there appears on each side of the coelomic wall just beneath the pronephric region, a longitudinal thickening of the peritoneum. Along the dorsal border of this thickening there is then proliferated a ridge of cells, whose edge grows downward and presently fuses with the ventral border of the thickening. In this manner a tube is formed, which, when completed, is held close to the body wall by a thin covering of the general peritoneum (Fig. 121). This process continues anteriorly to a point opposite the base of the lungs and posteriorly to the cloaca, which it reaches subsequent to metamorphosis. In the male this tube develops no further, and is very inconspicuous and without function, but is the rudiment of a *Müllerian duct* (see below).

In the Female. — The mesonephric duct is of course present in the female, but in this case acts only as a ureter. It possesses, nevertheless,

234 THE FROG: LATER OR LARVAL DEVELOPMENT

extremely slight enlargements, representing rudimentary seminal vesicles.

Each Müllerian duct or *oviduct*, on the other hand, develops as described in the male, but does not stop at the point there indicated. Instead, the rudimentary duct moves away from the body wall somewhat, though it still remains attached to that wall by its peritoneal covering. Between the duct and the wall the two layers of the covering then fuse

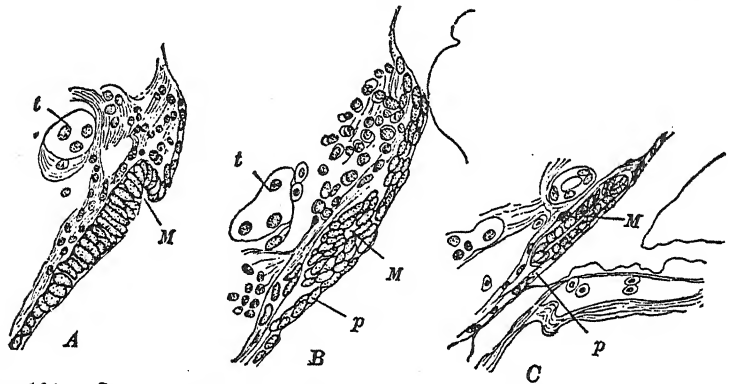


Fig. 121. — Sections through the developing Müllerian duct of a 34 mm. tadpole of *R. sylvatica*. From Kellicott (*Chordate Development*). After Hall. A. Section passing through the beginning of the Müllerian evagination. B. Section posterior to A. Duct established but still connected with peritoneum. C. Section still farther posterior, showing the separation of the duct from the peritoneum with which, however, it is covered. M. Müllerian duct. p. Peritoneum. t. Third pronephric tubule.

to form the mesentery-like sheet supporting the oviduct. Anteriorly the duct turns down slightly, and its end becomes dilated as the *infundibulum*, while posteriorly it acquires an opening into the cloaca; between these points it gradually becomes greatly convoluted and thickened.

THE GONADS

The Indifferent Period. — As the early stages of these organs are identical in the male and female, a single account will suffice for both.

At about the time of hatching, a slight median dorsal ridge appears on the outside of the enteron (Fig. 122, A). It is composed of primordial germ cells, which, as in other cases, have apparently arisen from among the cells of the gut. Indeed, at this time it is difficult to distinguish the cells of the ridge from the enteric cells below them. Presently, as noted above, the lateral plates of mesoderm press in toward each other in this region, and as they meet, they separate the ridge of cells

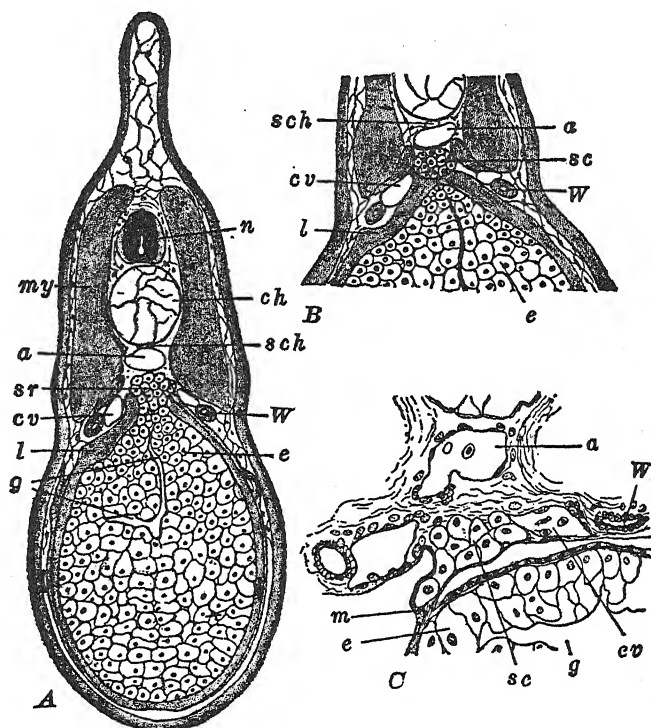


Fig. 122. — Sections showing the origin of the sex-cells (germ cells) in *R. sylvatica*. From Kellicott (*Chordate Development*). After Allen. A, B. Sections of a 7.5 mm. larva showing (A) sex-cell ridge of endoderm and (B) its separation as the sex-cell cord. C. Part of a section of an 8.3 mm. larva showing the beginning of the migration of the sex-cells, resulting shortly in the division of the sex-cell cord into two parts.

a. Dorsal aorta. ch. Notochord. cv. Posterior cardinal vein. e. Endoderm cells. g. Gut cavity. l. Lateral plate of mesoderm. m. Mesentery. my. Myotome. n. Nerve cord. sc. Sex-cell cord (not to be confused with sexual cords). sch. Subchordal rod (hypochorda). sr. Sex-cell ridge. W. Wolffian duct.

(sex-cell ridge) from the enteron, so that the former lies just dorsal to the newly formed mesentery (Fig. 122, B). This ridge, now the *sex-cell cord* (not to be confused with the sexual cords), soon divides in two longitudinally and each part moves a short distance ventro-laterally, taking up its position just beneath one of the cardinal veins. The two parts covered by coelomic epithelium (peritoneum) project slightly into the coelom in these regions and are known as the *genital ridges*. As each ridge increases in size it projects further into the body cavity in

236 THE FROG: LATER OR LARVAL DEVELOPMENT

which it is suspended by the peritoneal epithelium which covers it. This epithelium gradually presses in above the organ, and thus forms a double sheet of tissue similar to that which supports the oviduct. As noted in the description of the adult organ, this sheet in the case of the ovary is termed the *mesovarium* and in the case of the testis the *mesorchium*. At this stage sex is still indistinguishable, and the gonad

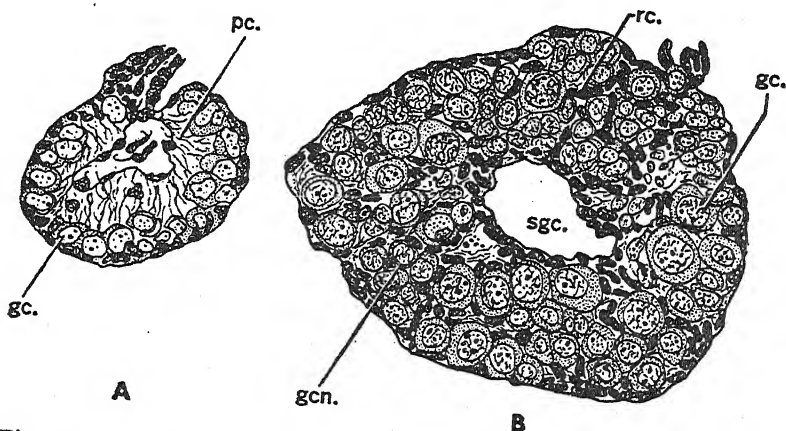


Fig. 123. — A. Section through the gonad of a 30 mm. tadpole of *R. catesbeiana*. B. Section through a young ovary from a tadpole of the same species. The secondary genital cavity lined with rete cord cells is small, but the germ cell nests of which the rest of the gonad is composed are already beginning to break up. After Swingle.

gc. Germ cell. gcn. Germ cell nest. pc. Primary genital cavity. rc. Rete cord cells. sgc. Secondary genital cavity or ovarian sac.

whether male or female consists simply of an elongated sac in which the germ cells are coming to be arranged about the periphery. Throughout the interior there exists a space which is filled by a jelly-like substance containing a few nuclei, and though thus occupied by jelly this region is termed the *primary genital cavity* (Fig. 123, A). The developing organ now occupies a position just beneath the mesonephros, from which cells have migrated to the base of the gonad. Here they produce strands, the *rete cords*, which grow ventrally into the primary genital cavity, and dorsally into the mesonephros (Witschi, '52).⁸ At this point in most Frogs the sexes begin to be differentiated as follows:

⁸ These strands are sometimes designated as the sexual cords, or sex cords (Swingle), but it seems preferable to reserve these terms for the strings of germ cells coming from the germinal epithelium, and found in many of the higher vertebrates (see Chick).

The Period of Sexual Differentiation.— In the case of a gonad destined to become an ovary the germ cells about the periphery begin to multiply. Simultaneously the masses of rete cord material which at certain points have grown down into the primary genital cavity begin to develop spaces within themselves. These new spaces within the rete cord material are known as the *secondary genital cavities*, and though at first occurring at intervals along the length of the organ they presently become more or less confluent. The larger cavities formed by this confluence are called *ovarial sacs*, whose walls composed of rete cord cells, are everywhere in contact with the innermost layer of germ cells. These germ cells soon become arranged in groups or nests, each nest being surrounded by a layer of follicular cells apparently derived from the peritoneum. Later the nests break up, and each growing oöcyte has its own follicle (Fig. 123, *B*). As this growth of the oöcytes and their follicles proceeds, their pressure upon the walls

of the ovarian sacs causes these walls to approximate one another until the cavities of the sacs are virtually obliterated. According to most accounts there always remain in the Frog a few nests of oögonia close against the periphery of the ovary, and from these are derived the new oöcytes for each breeding season.

In each gonad which is to form a testis on the other hand a different procedure occurs. The multiplication of the germ cells is less at first, while the proliferation of the rete cord material is greater. The latter also does not develop extensive secondary genital cavities as in the case

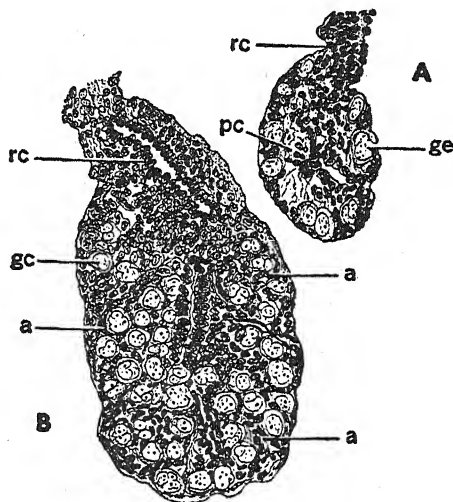


Fig. 124. — *A*. Section through a gonad of *R. catesbeiana* showing the first signs of a beginning testis. Note the rete cord material extending out among the germ cells, and the absence of any extensive secondary genital cavity. *B*. A developing testis from the same species showing nests of germ cells, the forerunners of ampullae, and eventually tubules. Near the hilus or base of the organ note the rete cords forming the distal parts of vasa efferentia, which lower down branch out to connect with the ampullae. After Swingle.

a. Ampullae. *gc*. Germ cell. *pc*. Primary genital cavity. *rc*. Rete cords.

238 THE FROG: LATER OR LARVAL DEVELOPMENT

of the ovary, but instead remains relatively condensed. Into this, germ cells from the periphery seem to migrate (*R. sylvatica*, Witschi, '29), or in some cases cords of rete material grow out and surround groups of the germ cells (*R. catesbeiana*, Swingle, '26, Fig. 124, *A*). In either event cysts are thus formed lined partly by rete material, and partly by connective tissue or stroma. These may at first be described as ampullae, but eventually they lengthen out to form the *seminiferous tubules* of the adult. Within a given tubule most of the germ cells are usually at the same stage of development, except that a few residual spermatogonia apparently always remain to furnish sperm for the next season. As indicated above the seminiferous tubules are connected with the vas deferens through the vasa efferentia, the latter being formed partly from the rete cords and partly from mesonephric tubules (Fig. 124, *B*).

In both sexes the anterior third or half of each genital ridge fails to develop as indicated above. Instead, some time previous to metamorphosis this portion of the organ starts to become converted into the *fat bodies*.

It may also be noted that while this is the normal situation in Frogs, in Toads an interesting modification occurs. In most species of the latter animal the male possesses a small ovary-like body lying between the testis and fat body. It is called *Bidder's organ*, and has long been an object of interest. It is now believed by some (Witschi, '33) to represent an incipient ovary. According to this view it is held that the undifferentiated gonad in this region is deficient in medullary substance, thus allowing the cortex here to develop to a limited degree. Though as indicated, it is most common in males, it also occurs in some female Toads where it again appears, according to this interpretation, as an undeveloped piece of the ovary (Witschi, '33).

SEX REVERSAL IN AMPHIBIA

The occurrence of hermaphroditism and of sex reversal is always of interest in any animal which normally has two distinct sexes. Hence since considerable experimental work in this connection has been done upon the Amphibia it is appropriate to say a few words about it at this point.

From the foregoing account of normal gonad development in the Frog, it is obvious that the gonads of the two sexes start out from common primordia. What then causes their differentiation? It will be recalled from statements in the chapter on the germ cells that the initial determination of sex in general is believed to depend on a balance be-

tween male and female determining genes. The female determining genes in most animals occur in the X-chromosome and the male determining genes in the autosomes. It was also noted, however, that these gene effects, like others, can be modified by the environment, and that the Amphibia afford good examples of this fact. The complete story here is not yet entirely clear, but experiments on both Frogs and Urodeles by Burns, Humphrey, Witschi and others seem to have elucidated the more essential factors. These experiments involve transplanting gonad primordia of various stages between animals of opposite sex, uniting at random many individuals to form pairs (parabiosis, Humphrey, '36), injecting sex hormones, and altering the temperature at critical stages. For example in the Frog the cortex of the partially differentiated gonads is apparently inhibited by excessive warmth (32 degrees C.), causing prospective ovaries to become testes (Witschi, '29). Or in various species of *Amblystoma* it was shown that the implantation of a gonad preprimordium of the opposite sex in a larval host of another stage shifts the sex of the implant or the host (Humphrey, '53). Also injection of male hormone, testosterone, during differentiation of prospective female gonads in *Amblystoma*, produces partial reversal to males, while injection of oestrone in prospective males causes reversal toward the female (Burns, '38, '39). Chang, '53, however, thinks substances other than these hormones are involved. Lastly Bruner and Witschi, '54, showed that early use of testosterone actually causes the pre-medullary component of the prospective male gonad to form mesonephric tubules instead of medulla, without which the cortex partly differentiates into ovary.

Without going into detail the conclusions suggested by the results of these procedures may be summarized as follows: The chromosome complex gives the first impetus to sex determination, apparently by affecting the character of the mesoderm at the gonad site (gonad preprimordium). The character of this preprimordium having been thus initially influenced then determines whether, in the seemingly indifferent gonad rudiment arising from it, the cortex or the medulla shall acquire the ascendancy. As soon as one or the other of these tissues does gain a start it begins to produce a substance with two effects. One effect is to stimulate still further the development of the favored tissue, cortex or medulla, and the other effect is to inhibit the development of the opposite tissue. Thus when once initiated the general result is cumulative. Finally when the mature gonad has formed, it produces the usual sex hormones, testosterone or oesterone, and these tend to control such secondary sex characters as may be characteristic of the species. With this

240 THE FROG: LATER OR LARVAL DEVELOPMENT

history in mind we may better understand various types of sex anomalies in animals possessing a perfectly normal chromosome complex. Thus it is possible to have complete sex reversal in both gonads and gonoducts, or there may be partial reversal in these organs giving a sort of nondescript neuter. Also there may be complete reversal on one side only, resulting in real hermaphrodites. Lastly there may be reversal in parts of both gonads producing a kind of sex mosaic.

INTERNAL DEVELOPMENT: THE SKELETON

Only a brief outline of the development of the specific parts of this system as it occurs in the Frog, Chick and Pig will be given in this text. For further details the reader is referred to more extended accounts cited in the bibliography. However, since the general histogenesis of the different types of bone is essentially similar in all true Vertebrates, it seems desirable to give some details concerning the basic processes involved. This will therefore be done at this point, with the understanding that though the fundamental pattern is similar in all the forms studied there are some variations in detail. The more important of the latter will be indicated in connection with the forms concerned.

THE HISTOGENESIS OF BONE

Dermal or Membrane Bone. — This type of bone is peculiar in that ossification (deposition of calcium salts) occurs directly within membranous connective tissue without the intervention of a cartilaginous stage. It is a method of bone formation which occurs extensively, though not exclusively, as we shall see, in certain bones of the skull, and may be described as follows:

Within a connective tissue layer where the bone is to form, certain undifferentiated mesenchymal cells become arranged in isolated strands, each strand being several cells in thickness. These cells then lose their fine processes characteristic of the cells of mesenchyme, and begin to secrete in their midst a delicate fiber, for which reason they are termed *fibroblasts*. The fiber they secrete is called an *osseine fiber*, but is not essentially different from other nonelastic or white connective tissue fibers consisting of collagen. Soon numerous fibers thus formed in a particular region come to constitute a thickened strand. In the next step the fibroblast cells which deposited the fibers become modified chemically, and about each fiber they begin to deposit calcium salts. When this stage has been reached the cells involved are called *osteoblasts*.

The fibroblasts and osteoblasts, continuing to form respectively both ossein fibers and calcium salts about each original strand, add to its thickness and length. As a consequence of the latter type of growth, these thickened and ossified strands, now termed *trabeculae*, are brought into contact with each other, and thus a bony network is produced (Fig. 125). Since, moreover, the deposition of fibers and calcium (matrix) is more or less periodic we find any given trabecula consisting of layers of bone somewhat like the growth rings of a tree. It should

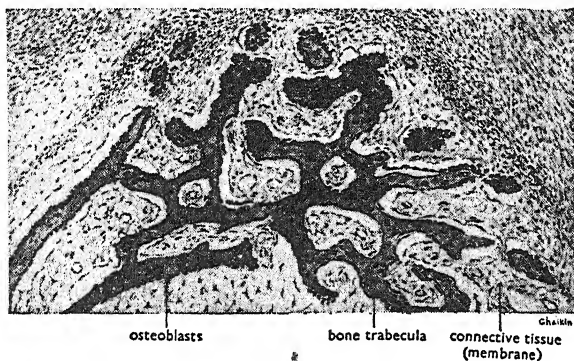


Fig. 125.—Trabeculae of a piece of membranous jaw bone from a Mammal in the process of being thickened by fibroblasts and osteoblasts. Drawing from Turtlox preparation.

also be obvious that as the osteoblasts deposit their matrix they must keep moving away from the original center of deposition or else be imprisoned in their own products. As a matter of fact different ones do both these things. Those which move, and thus remain at the surface continue to function as osteoblasts. Those which are trapped, so to speak, cease deposition, but do not die. They remain as permanent bone cells with delicate processes extending out into the matrix. These processes convey nutriment from the spaces containing blood vessels to the cell bodies, which furnish it to the organic ossein fibers. When these cells and the fibers deteriorate and finally disappear with senescence only the calcium salts are left, and the bone becomes brittle.

The bone formed as described evidently contains many irregular spaces, and so long as these exist it has a spongy texture. In the central parts of membrane bones which are under discussion this condition is permanent, and the bone is known as *cancellous*. The spaces in such bone, however, are not empty. They are filled with blood vessels and large thin walled sinusoids, surrounded and supported by reticulate

connective tissue (stroma). The stroma contains all types of mature blood corpuscles which are being constantly produced by its undifferentiated cells, and passed as needed into the sinusoids, which communicate with the blood vessels. This conglomeration of loose connective tissue, blood spaces, vessels and cells is termed *marrow*. Sometimes it is permeated with fat containing cells, and is then known as yellow marrow as compared with the corpuscle producing red marrow. The spaces thus occupied by marrow of one sort or the other are lined by a more dense flat connective tissue layer, now containing fibroblasts and osteoblasts, and known as the *endosteum*. It is not to be assumed of course that marrow exists only in dermal bones. It occurs as much or more in the other type of bone as will presently be pointed out.

As so far described it might be supposed that dermal bone is entirely cancellous, but this is not the case. Surrounding the first formed cancellous material is a layer of connective tissue similar to the endosteum which comes to line the marrow spaces. This being outside, however, is called *periosteum*, and it also contains fibroblasts and osteoblasts. These fibroblasts and osteoblasts, like those of the endosteum covering the trabeculae, deposit fibers and bone, in this case in continuous layers completely surrounding the cancellous bone and marrow. Thus is formed one type of *compact* bone, between whose layers entrapped bone cells occur at intervals, just as in the case of the layers deposited on the trabeculae. As implied, however, this is not the only type of compact bone that may be formed. In some cases, as will be described more in detail below, some of the more outer marrow spaces are filled with concentric bone layers which thus make the region so involved compact. More will presently be said of this method of forming compact bone. Also curiously enough some of the first continuous peripheral layers deposited may prove not to be permanent. Another type of connective tissue cells, known as *osteoclasts*, may invade this peripheral bone and eat out cavities in it so that it in turn becomes cancellous. Later, however, such secondary cavities will be filled in again in the manner noted in the case of the other cancellous bone, thus making it again compact. In any case a few of the continuous peripheral layers are always finally left surrounding the entire bone. The end result of all these processes is that the completed dermal bone consists of a cancellous and marrow filled central region surrounded by varying thicknesses of compact layers of one sort or another. Bones of this type it should be added are more or less flat in shape, occurring for the most part, as noted, as covering bones of the skull.

Cartilage or Endochondral Bone.— In the case of bone of this type, which comprises the larger part of the skeleton, ossification does not occur directly from membrane, but from an intervening cartilaginous stage. The process is as follows:

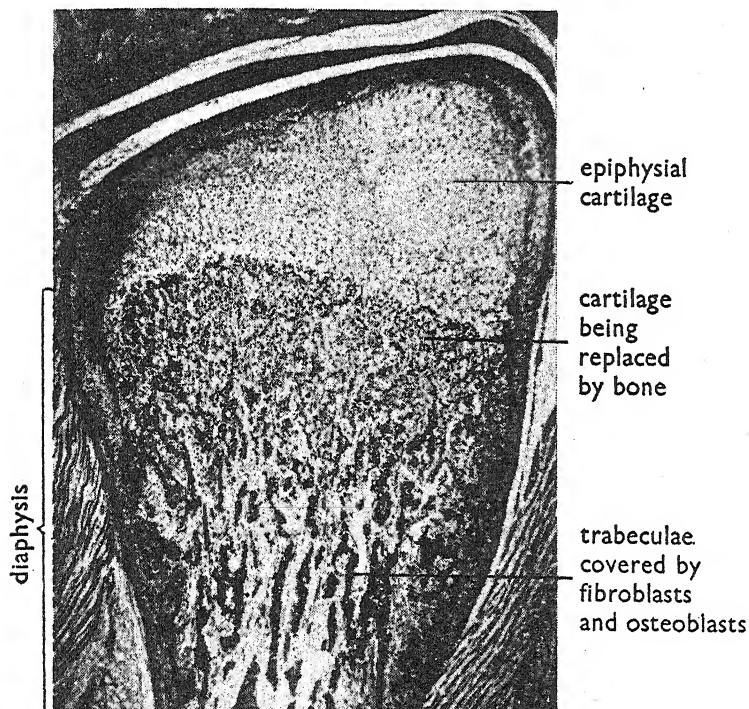


Fig. 126. — The epiphysis and a portion of the diaphysis of a developing mammalian long bone. The epiphysis is still entirely cartilaginous. At the boundary between the two regions, however, the cartilage is being reduced to fine strands by means of chondroclasts. Further down in the diaphysis these strands are being built up into bony trabeculae by the fibroblasts and osteoblasts which cover their surfaces. Photo of a Turttox preparation by the author.

As before the initial condition is that of a mass or layer of mesenchyme. The mesenchymal cells then lose their processes much as in the preceding case. Now, however, instead of becoming aggregated in strands they form a densely packed mass of multiplying cells which gradually assumes the shape of the future bone. These cells, however, do not form bone. Instead each cell begins to secrete a gelatinous matrix of a substance called *chondrin*. This is at first quite elastic, and thus the cells are able to move away from each other as they secrete.

Later the chondrin condenses to form the mature *cartilage matrix*. When this stage is reached, the cells can no longer push each other apart, or multiply much. Each cell may divide once or twice, and the small group secretes just enough to cause the cartilage immediately around it to become especially dense. Thus we have formed a mass of cartilage the shape of the future bone. It consists of a dense chondrin matrix containing numerous small groups of cells. Finally this mass of cartilage has surrounding it a firm connective tissue layer called *perichondrium*, whose cells, like those of the periosteum, continue for a time to add to the cartilage peripherally. The next step is the destruction of the cartilage and its replacement by bone.

The destruction of the cartilage is brought about by the same cells which previously deposited it. Now, however, these cells behave like the osteoclasts noted above, only in this case they act as *chondroclasts*, and erode cartilage instead of bone. They proceed in such a way that soon they have reduced the cartilage to delicate strands whose surfaces they cover. Meanwhile certain cells of the perichondrium become active and, along with blood vessels, start to invade the disappearing cartilage. These cells turn out to be fibroblasts and osteoblasts which soon replace the cartilage eroding cells surrounding the cartilaginous strands. These cartilaginous strands thus take the place of the fibrous strands of cancellous membrane bone, and around them the new fibroblasts and osteoblasts deposit fibers and calcium salts to form cancellous *endochondral bone* (Fig. 126). The resulting bony trabeculae surrounding marrow filled spaces are the same as before, only in this instance the bone was preceded by cartilage. In view of its behavior the surrounding perichondrium is from now on termed periosteum. This endochondral cancellous bone may now become compact in the same way that the cancellous bone of membranous origin does so. The details of that process, which were merely suggested previously, are as follows:

The bone forming cells, fibroblasts and osteoblasts, covering the trabeculae gradually so arrange themselves while depositing bone that the marrow spaces become tube shaped. Then as the osteoblasts and fibroblasts continue to deposit layers of calcium salts and fibers, part of the cells withdraw toward the center of the constantly decreasing marrow space. Others, as previously described in another connection, are trapped between the layers to form permanent bone cells. In this manner concentric layers of bone are produced surrounding a marrow space which finally is reduced to a small canal containing only a couple of blood vessels and a few cells. This is called an *Haversian canal*, and to-

gether with the concentric arrangement of the bone layers about it constitutes an *Haversian system* (Fig. 127). Compact bone so formed therefore would consist of many such systems filling completely the spaces between the original trabeculae. The canals of the numerous systems are, moreover, interconnecting, so that the blood vessels in them ultimately reach the periosteum on the one hand or the central marrow on the other.

It should again be emphasized that the actual process of bone depo-

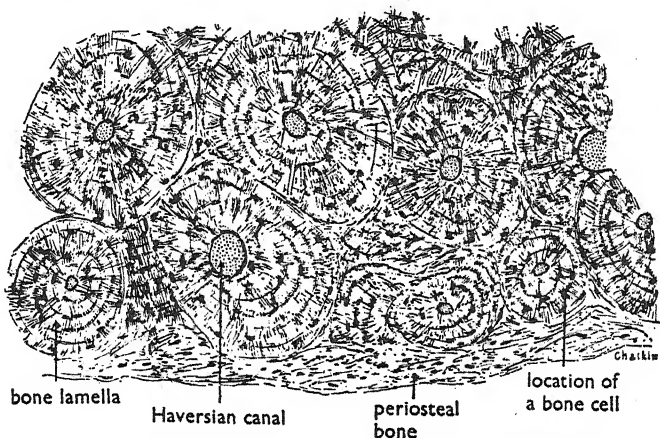


Fig. 127. — Haversian systems from a section of adult bone.

sition just indicated as occurring in compact endochondral bone is exactly the same as that referred to in the case of one type of compact membrane bone. The difference is entirely in the preceding processes. In the former case the compact bone was preceded simply by cancellous bone. In the present case the cancellous bone was itself preceded by cartilage. In addition to this difference in the method of development between membrane bones and the part of all endochondral bones thus far described, there is one other feature characteristic of the final structure of most of the latter. A good deal, or all, of the central cancellous material in mature endochondral bones is usually removed entirely by osteoclasts, and the relatively large single space so produced occupied by the marrow. Any other marrow in such bones will, as in membrane bones, occupy the spaces of any cancellous bone which remains (Figs. 126, 128).

It must now be added that even so called endochondral bones are not entirely so. This is because the endochondral compact bone formed in

the manner we have indicated is always ultimately surrounded by bone formed directly from the periosteum, and hence entirely membranous in origin. This may involve simply the laying down of the final circumferential layers. Usually, however, as in the case of completely membranous bone, some of the early surrounding layers are rendered can-

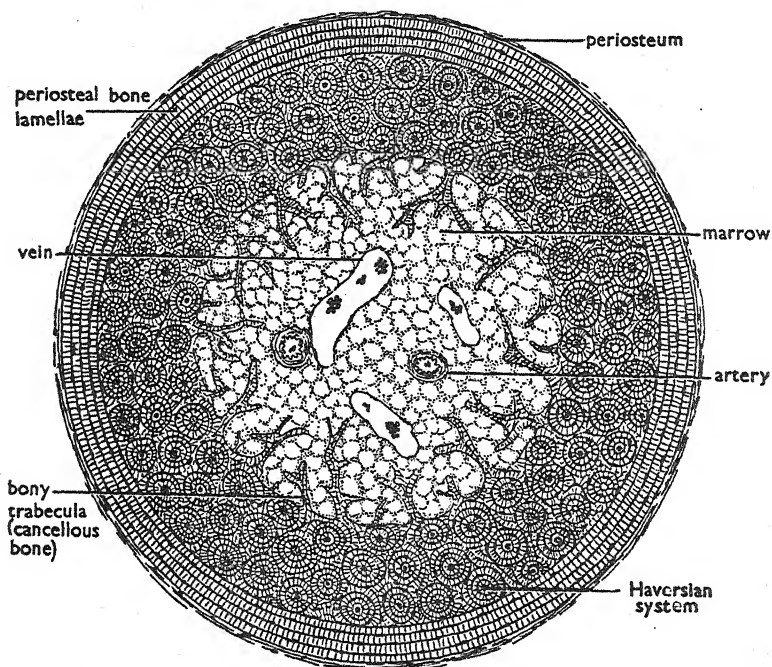


Fig. 128. — A semi-diagrammatic representation of a cross section of a mammalian long bone (endochondral), showing periosteum, periosteal bone lamellae, Haversian systems and marrow.

cellous by osteoclasts, with the subsequent development of Haversian systems. And in this case the latter were obviously not immediately preceded by cartilage. Thus it is to be remembered that when, in later discussions, we refer to certain bones as being endochondral in origin, it is only a part of such bones which were really preformed in cartilage. So-called "membrane bones" are, however, entirely preformed in membrane.

Finally it should be noted that in the case of any kind of bone the later stages in its formation involve a very intimate connection with the periosteum. This is because that, in addition to blood vessels, innumerable white periosteal connective tissue fibers are surrounded by

the final calciferous deposits. Thus these fibers, known as the *fibers of Sharpey*, are directly continuous from the periosteum right into the compact bone forming an extremely tight union between connective tissue and the bone itself. It may also be noted that at certain points these fibers are aggregated into bundles called *tendons* which are continuous in the opposite direction from the bone into the connective tissue sheaths of its muscles. We are now prepared to turn to a brief consideration of the formation of the various parts of the skeleton of the Frog.

THE VERTEBRAL COLUMN

At or a little before the time of hatching, the skeletogenous sheath has already come to surround the notochord and nerve cord, as indicated above. Some time after hatching (about 15 mm.), cartilage develops within this sheath and presently becomes divided into sections corresponding in position and number to the future vertebrae. Within each such section, moreover, the cartilage about the chorda soon forms a ring which completely surrounds it (Fig. 129). Within these cartilaginous rings, ossification now starts and gradually spreads inward until the notochord at the core of every ring is entirely obliterated. Thus is formed the *centrum* of each vertebra. Meanwhile between these vertebral centra the notochord is also obliterated by the ingrowth of cartilage. Each intervertebral disc thus developed, later splits into an anterior and a posterior part. Finally, during metamorphosis each of these parts becomes ossified and fused with the end of the contiguous centrum.

In a like way the *neural arches* ossify from cartilage which extends dorsad from the centra around the nerve cord, while the *transverse processes* arise as bits of cartilage projecting laterally from each centrum, which also later ossify. Eventually minute cartilaginous ribs form at the ends of the processes, but are soon fused with the latter. Vertebra formation is induced by nerve cord rather than notochord (Holtzer, '52).

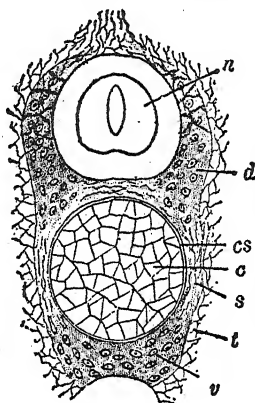


Fig. 129. — Transverse section through the vertebral column in the body region of a larva of *Xenopus capensis*. From Kellcott (*Chordate Development*). After Schauinsland.
c. Notochord. d. Dorsal vertebral cartilaginous arch. s. Sclerotomal (skeletogenous) sheath. n. Nerve cord. cs. Chorda sheath (primary and secondary). t. Perichondral connective tissue. v. Ventral (hypochordal) vertebral cartilage. The dorsal and ventral cartilaginous elements have not yet come to surround the notochord.

248 THE FROG: LATER OR LARVAL DEVELOPMENT

As already noted, the Frog possesses only nine real vertebrae, and the above description applies only to them. The skeletogenous elements of the last two somites, however, form a single tubular piece of cartilage which surrounds the end of the notochord. Later it also becomes mostly ossified, and is known as the *urostyle*.

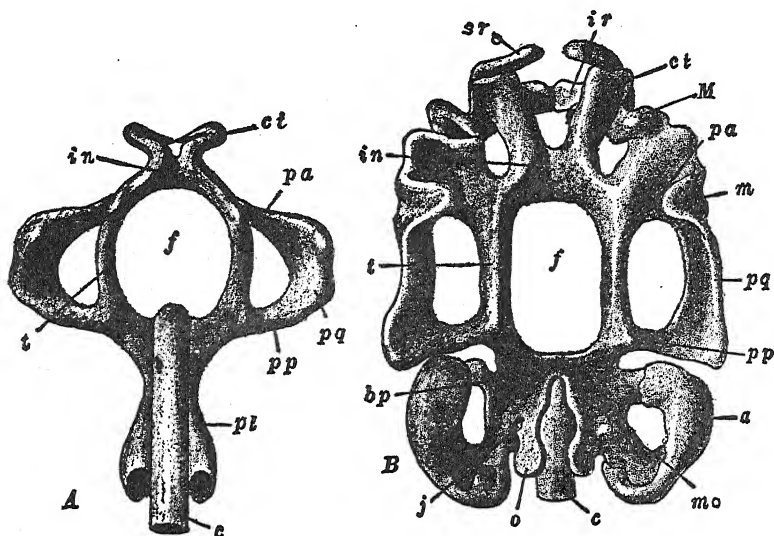


Fig. 130. — Dorsal views of the chondrocranium of the Frog larva. A. Chondrocranium of a 7.5 mm. larva of *R. temporaria*. From Kellicott (*Chordate Development*). After Gaupp, from Stöhr-Ziegler model. B. Chondrocranium of a 14 mm. larva of *R. fusca*. After Gaupp, from Ziegler model.

a. Auditory capsule. *bp*. Basal plate. *c*. Notochord. *ct*. Trabecular cornu. *f*. Basiscranial fontanelle. *in*. Internasal plate. *ir*. Infrarostril cartilage. *j*. Jugular foramen (for IX and X cranial nerves). *m*. Muscular process. *M*. Meckel's cartilage. *mo*. Mesotic cartilage. *o*. Occipital process. *pa*. Anterior ascending process of palatoquadrate cartilage. *pl*. Parachordal plate. *pp*. Posterior ascending process of palatoquadrate cartilage. *pq*. Palatoquadrate cartilage. *sr*. Suprarostril cartilage. *t*. Trabecular cartilage.

THE SKULL

The Floor. — The posterior portion of the skull floor, i.e., that part which lies beneath the hind brain, is formed medially by the notochord. On each side of the notochord a cartilaginous rod develops which fuses with the chorda or rather with the cartilage which soon takes its place, thus completing the floor in this region. These rods are called the *parachordals*, and the fused mass is the *parachordal plate* (Fig. 130, A).

In front of each parachordal is another rod. These rods are curved

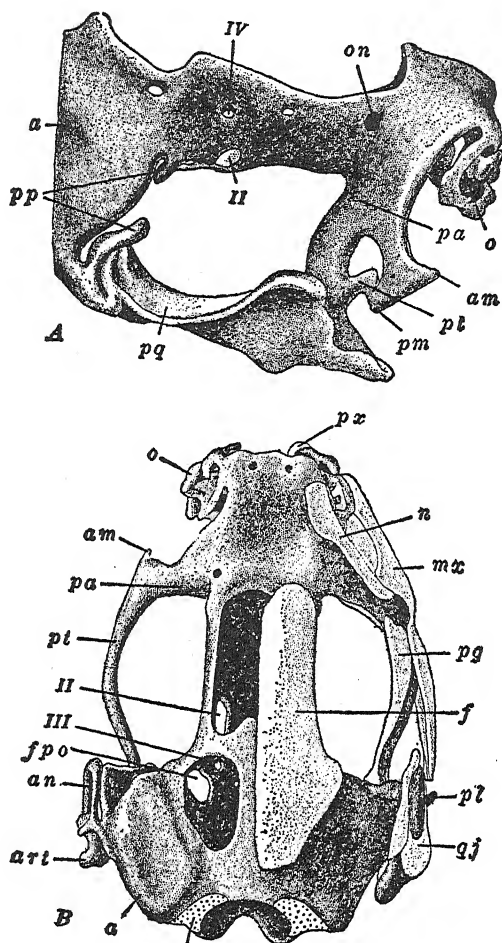


Fig. 131. — *A*. Anterior portion of chondrocranium of *R. fusca* during metamorphosis. Lateral view. From Kellicott (*Chordate Development*). After Gaupp, from Ziegler. *B*. Skull of a 2 cm. *R. fusca*, after metamorphosis. Dorsal view. Membrane bones removed from left side. After Gaupp, from Ziegler.

a. Auditory capsule. *am*. Anterior maxillary process. *an*. Annulus tympanicus. *art*. Articular process of palato-quadrate cartilage. *eo*. Exoccipital bone. *f*. Frontoparietal bone. *fpo*. Prootic foramen. *mx*. Maxillary bone. *n*. Nasal bone. *o*. Olfactory cartilages. *on*. Orbito-nasal foramen. *pa*. Anterior ascending process of palato-quadrate. *pg*. Pterygoid bone. *pl*. Plectrum. *pm*. Posterior maxillary process. *pp*. Posterior ascending process of palato-quadrate. *pq*. Palato-quadrate cartilage. *pt*. Pterygoid process of palato-quadrate. *px*. Premaxillary bone. *qj*. Quadratojugal bone. *II*. Foramen for optic nerve. *III*. Foramen for III cranial nerve. *IV*. Foramen for IV cranial nerve.

somewhat, with their concave sides facing each other, and their posterior ends fused with the anterior ends of the parachordals. Their own anterior ends grow toward each other and fuse between the olfactory organs; these rods are the *trabeculae*. The space between them in the anterior floor of the skull is the *basicranial fontanelle*, which temporarily lodges the infundibulum. Later, as the trabeculae grow together, this opening is closed.

The Sides, End, and Roof. — The floor has reached the stage indicated only a short time after hatching. The other cartilaginous parts of the skull then develop as follows:

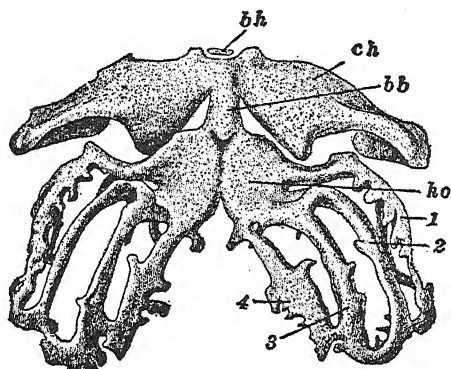


Fig. 132. — Hyoid and branchial arches of a 29 mm. larva of *R. fusca*. Ventral view. From Kellcott (*Chordate Development*). After Gaupp, from Ziegler.

bb. Basibranchial (first), or copula. bh. Basihyal. ch. Ceratohyal. ho. Hypobranchial plate. 1-4. First to fourth ceratobranchials.

In the posterior region the cartilaginous auditory capsules appear at the sides of the head (Fig. 130, B). Ventrally they are presently united with the skull floor by the *mesotic* and *occipital cartilages*. The capsules thus form the sides of the posterior part of the skull, while the occipital cartilages grow up to form the posterior walls and the roof of this region. Between the occipitals is a posterior opening, the *foramen magnum*, through which the spinal cord passes into the brain.

Anteriorly the trabeculae grow up to form the sides of the skull in the neighborhood of the orbits. Their more anterior portions then grow together dorsally forming the anterior roof. Between this anterior roof and the posterior one formed by the occipitals is the *supra-cranial fontanelle*. The extreme anterior ends of the trabeculae go to form the *olfactory capsules*, which are partly separated from the brain cavity by a septum. All of these changes, both anterior and posterior, are virtually completed in larvae of 3 cms.

Dermal Elements in the Skull. — The cartilaginous skull thus formed later becomes ossified, in the usual way. Before this occurs, however (about 20 mm.), many of the parts begin to be covered by

bony plates originating in the dermis (in the manner indicated above) and hence called *dermal bones* (Fig. 131). Some of these plates, such as the *fronto-parietals*, serve to cover open spaces left in the cartilage, e.g., the supra-cranial fontanelle. Most of the dermal bones as well as those formed in the cartilage have appeared before metamorphosis is complete.

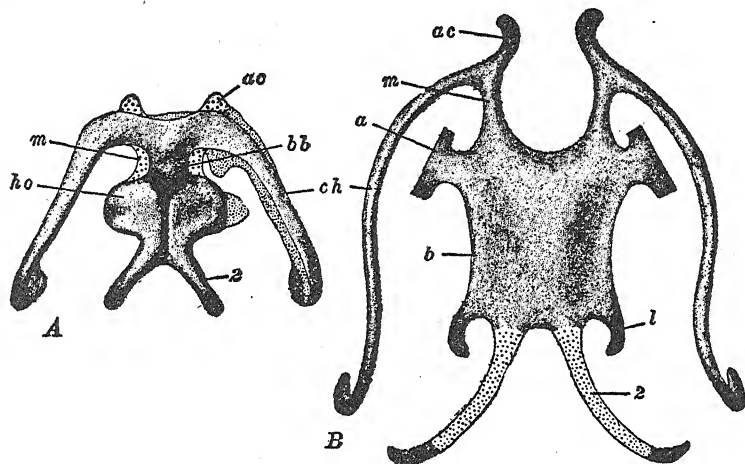


Fig. 133. — *A*. Hyobranchial apparatus of *R. fusca*, toward the end of metamorphosis. The left side is shown in a more advanced stage than the right, in that less cartilage is present. The original cartilage is indicated by fine stipples. The coarse stipples indicate the cartilage added during the early part of metamorphosis. From Kellicott (*Chordate Development*). After Gaupp, from Ziegler. *B*. Hyobranchial apparatus of a 2 cm. *R. fusca*, after metamorphosis. After Gaupp, from Ziegler.
a. Alar process. *ac*. Anterior process of hyoid cornu. *b*. Body of hypobranchial cartilage. *bb*. Basibranchial (first), or copula. *ch*. Ceratohyal (hyoid cornu in *B*). *ho*. Hypobranchial plate. *l*. Postero-lateral process of hypobranchial cartilage. *m*. Manubrium. 2. Remains of second ceratobranchial (postero-medial process of hypobranchial cartilage).

The Visceral Arches. — These arches at first consist merely of concentrations of mesoderm, as indicated above. Shortly after the mouth opens, however, all have developed skeletal elements of cartilage. The cartilage of the mandibular arch early becomes divided into a dorsal portion, the *palato-quadrate*, and a ventral portion, *Meckel's cartilage*. The former becomes fused anteriorly and posteriorly with the trabeculae and at metamorphosis is considerably modified to form a part of the upper jaw. As noted above, furthermore, a small outgrowth becomes separated from the posterior or quadrate portion of this cartilage and gives rise to the annulus tympanicus of the middle ear. Meck-

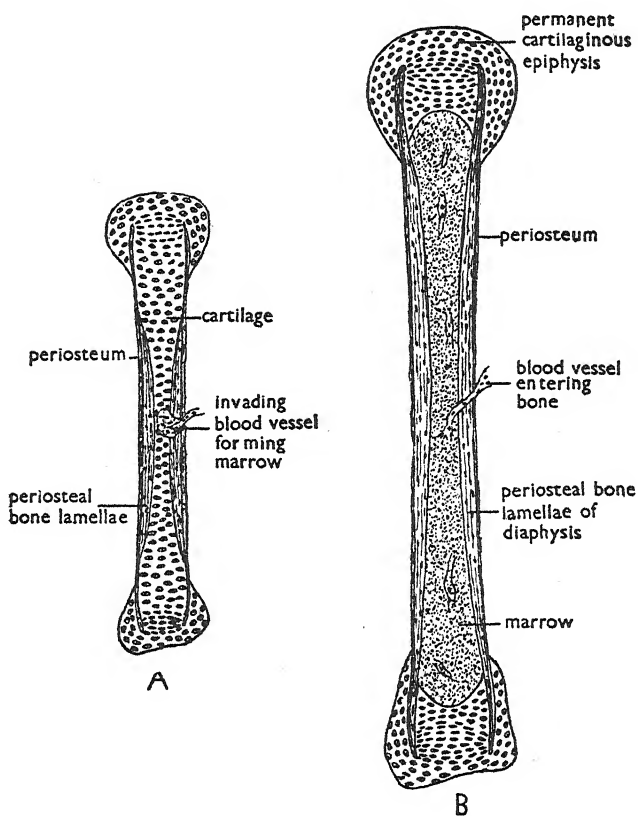


Fig. 134. — Semi-diagrammatic representations of medial longitudinal sections of growing long bones of Bullfrog tadpoles. *A*. A young stage in which cartilage is still the dominant element in both diaphysis and epiphysis. In the diaphysis, however, the periosteum has already replaced some of the cartilage with circumferential bony lamellae. Also a blood vessel along with chondrioclasts has invaded the cartilage, and is beginning to form the marrow. *B*. A later stage in which the diaphysial cartilage has all been replaced by marrow and circumferential bone lamellae laid down by the periosteum. Note that in this case there are not, and never would have been, any Haversian systems, all the bone of the diaphysis being formed from periosteal lamellae. The epiphyseal cartilages, at this and the preceding stages, contain a lozenge-shaped growing zone characteristic of the Frog. The epiphyses remain permanently cartilaginous in this animal. After studies by Marvin.

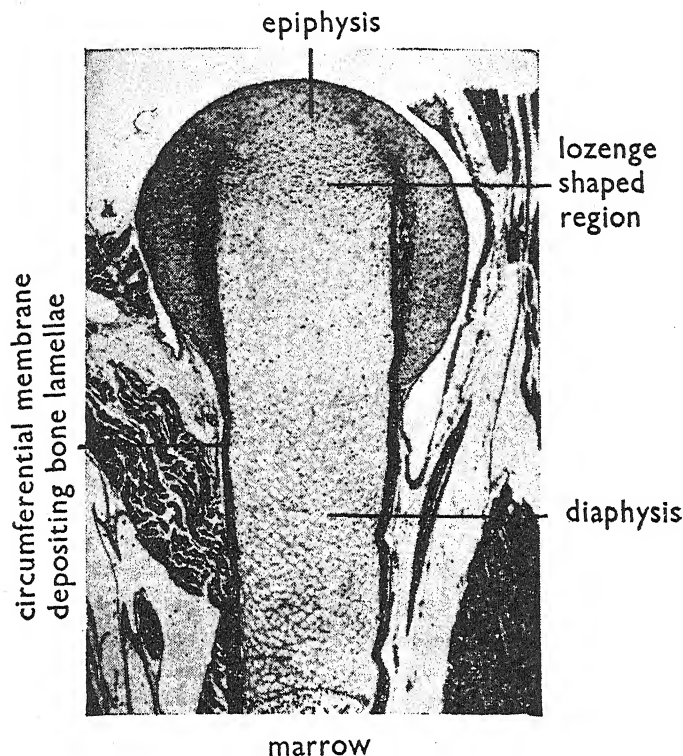


Fig. 135. — The epiphysis and part of the diaphysis of a developing Bullfrog femur in a condition similar to that diagrammed in Fig. 134, *A*. Note the cap of epiphyseal cartilage extending down on either side of the diaphysis. Also in this cap note the lozenge-shaped region of dividing cells. On each side of the diaphysis the heavy lines represent dense circumferential connective tissue within which the layers (lamellae) of circumferential bone are about to form. A small region of marrow which occupies the middle portion of the bone shows at the lower edge of the picture. (Author's photograph of preparation by Marvin.)

el's cartilage remains small throughout larval life, but constitutes the core of the lower jaw in the adult.

The hyoid arch (*Ceratohyal*) and the second branchial arch, together with certain median elements, form the *hypobranchial apparatus* of the adult. In the latter the hyoid arch becomes the so-called *hyoid* (greater) *cornu* or horn, while the second branchial arch becomes the *lesser cornu*. All of the other arches disappear entirely at metamorphosis (Figs. 132, 133).

THE APPENDICULAR SKELETON

Both the *pectoral* and *pelvic girdles* are said to be endochondral in origin, with the exception of the *clavicle*, which as in other animals is a membrane bone. The long bones of the limbs are also usually thought of as endochondral, but in the Frog, unpublished investigations by R. W. Marvin ('47) in the author's laboratory would seem to show that in a strict sense they are not so at all. In the case of these bones in this animal what appears to occur is this:

A cartilaginous core as usual first replaces the condensed mesoderm or membrane, and around this the bone is later laid down exclusively by the periosteum in circumferential layers (Fig. 134). The cartilage is then removed, as well as some of the first formed inner layers of bone. This removed material, however, is all replaced by marrow, none of it by bone. Hence if this account is correct there is no true endochondral bone involved, i.e., none which replaces cartilage or bone preceded by cartilage in the manner described above. The situation as so far indicated refers only to the bone shaft, i.e., the part defined in all such bones as the *diaphysis*. The condition at the ends, which are known as the *epiphyses*, remains to be discussed. In the case of the Frog the ends of the cartilaginous cores of the shaft of a long bone never become ossified at all, even after all growth has ceased. Thus the ends or epiphysis in this case consist of permanent caps of cartilage whose borders extend down somewhat over the bony cylinders which constitute the diaphysis (Fig. 135). These procedures in both diaphysis and epiphyses are at variance, as we shall see, with what occurs in both the Bird and the Mammal, which also differ somewhat from each other.

REFERENCES TO LITERATURE

CHAPTERS IV, V, VI

- Adelmann, H. B., "Experimental Studies on the Development of the Eye. III. The Effect of the Substrate ('Unterlagerung') on the Heterotopic Development of Median and Lateral Strips of the Anterior End of the Neural Plate of *Amblystoma*," *Jour. Exp. Zool.*, LVII, 1930. — "The Effect of the Partial and Complete Excision of the Prechordal substrate on the Development of the Eyes of *Amblystoma punctatum*," *Jour. Exp. Zool.*, LXXV, 1937.
- Albaum, H. G. and Nestler, H. A., "Xenoplastic Ear Induction between *Rana pipiens* and *Amblystoma punctatum*," *Jour. Exp. Zool.*, LXXV, 1937.
- Allen, B. M., "An Important Period in the History of the Sex-Cells of *Rana pipiens*," *Anat. Anz.*, XXXI, 1907. — "The Response of *Bufo* Larvae to Different Concentrations of Thyroxin," — "The Dominant Role of the Pars

- Anterior of the Hypophysis in Initiating Amphibian Metamorphosis," *Anat. Rec.*, LIV, 1932.
- Assheton, R., "On the Development of the Optic Nerve of Vertebrates, and the Choroidal Fissure of Embryonic Life," *Q. J. M. S.*, XXXIV, 1892. — "On the Growth in Length of the Frog Embryo," *Q. J. M. S.*, XXXVII, 1894. — "On the Growth Centers in Vertebrate Embryos," *Anat. Anz.*, XXVII, 1905.
- Atwell, W. J., "Effects of Thyreotropic and Adrenotropic Principles on Hypophysectomized Amphibia," *Anat. Rec.*, LXII, 1935.
- , and Holley, E., "Extirpation of the pars Intermedia of the Hypophysis in the Young Amphibian with Subsequent Silvery Condition and Metamorphosis," *Jour. Exp. Zool.*, LXXIII, 1936.
- Bacon, R. L., "Self-Differentiation and Induction in the Heart of Amblystoma," *Jour. Exp. Zool.*, LXLVIII, 1945.
- Barth, L. G., "Neural Differentiation without Organizer," *Jour. Exp. Zool.*, LXXXVII, 1941.
- , and Graft, S., "The Chemical Nature of the Amphibian Organizer," *Cold Spring Harbor Symp. on Quant. Biol.*, VI, 1938.
- Bautzmann, H., "Experimentelle Untersuchungen zur Abgrenzung des Organisationszentrums bei Triton taeniatus," *Arch. Entw.-mech.*, CVIII, 1926.
- Beckwith, C. J., "The Effect of the Extirpation of the Lens Rudiment on the Development of the Eye in Amblystoma punctatum, with Special Reference to the Choroid Fissure," *Jour. Exp. Zool.*, XLIX, 1927.
- Brachet, A., "Recherches sur l'ontogénèse des Amphibiens urodèles et anoures. (*Siredon pisciformis*—*Rana temporaria*)," *Arch. Biol.*, XIX, 1902. — "Recherches sur l'origine et l'appareil vasculaire sanguin chez les Amphibiens," *Arch. Biol.*, XIX, 1903. — "Gastrulation et formation de l'embryon chez les Chordés," *Anat. Anz.*, XXVII, 1905. — "Recherches expérimentales sur l'œuf de *Rana fusca*," *Arch. Biol.*, XXI, 1905 (1904). — "Recherches expérimentales sur l'œuf non segmenté de *Rana fusca*," *Arch. Entw.-mech.*, XXII, 1906. — "Recherches sur l'ontogénèse de la tête chez les Amphibiens," *Arch. Biol.*, XXIII, 1908. — "Recherches sur l'influence de la polyspermie expérimentale dans le développement de l'œuf de *Rana fusca*," *Arch. Zool. Exp.*, VI, 1910. — "Études sur les localisations germinales et leur potentialité réelle dans l'œuf parthénogénétique de *Rana fusca*," *Arch. Biol.*, XXVI, 1911. *Traité D'Embryologie de Vertébrés*, Paris, 1921.
- Burns, R. K., "The Effects of Crystalline Sex Hormones on Sex Differentiation in Amblystoma. I. Estrone," *Anat. Rec.*, LXXI, 1938. — "II. Testosterone propionate," *Anat. Rec.*, LXXIII, 1939.
- Cameron, J. A., "Primitive Blood-Cell Generations in Amblystoma," *Jour. Morph.*, LXVIII, 1941.
- Copenhaver, W. M., "Experiments on the Development of the Heart of Amblystoma punctatum," *Jour. Exp. Zool.*, XLIII, 1926. — "Initiation of Beat and Intrinsic Contraction Rates in the Different Parts of the Amblystoma Heart," *Jour. Exp. Zool.*, LXXX, 1939. — "Liver Extirpation and Implantation in Amblystoma Embryos with Particular Reference to Blood Formation," *Am. Jour. Anat.*, LXXIII, 1943. — "Heteroplastic Transplantation of the Sinus Venosus between Two Species of Amblystoma," *Jour. Exp. Zool.*, C, 1945.
- Cornman, I. and Grier, N., "Refraction of Light by Amphibian Egg Jelly," *Copeia*, 1933.
- Detwiler, S. R., "An Experimental Study of Spinal Nerve Segmentation in Amblystoma with Reference to the Plurisegmental Contribution to the Brachial Plexus," *Jour. Exp. Zool.*, LXVII, 1934. — "Further Observations upon Abnormal Growth Responses of Spinal Nerves in Amblystoma Embryos," *Jour.*

- Exp. Zool.*, LXIX, 1934. — "The Development of Spinal Ganglia following Transplantation of the Spinal Cord with or without Somites," *Anat. Rec.*, LXI, 1935. — "Growth Responses of Spinal Nerves to Grafted Brain Tissue," *Jour. Exp. Zool.*, LXXIV, 1936. — "Substitution of Lateral for Axial Mesoderm in Relation to the Development and Segmentation of Spinal Ganglia," *Jour. Exp. Zool.*, LXXVI, 1937. — "Observations upon the Migration of Neural Crest Cells, and upon the Development of the Spinal Ganglia and Vertebral Arches in Amblystoma," *Am. Jour. Anat.*, LXI, 1937. — "Does the Developing Medulla Influence Cellular Proliferation within the Spinal Cord," *Jour. Exp. Zool.*, LXXVII, 1937.
- DuShane, G. P., "Neural Fold Derivatives in the Amphibia. Pigment Cells, Spinal Ganglia and Rohon-Beard Cells," *Jour. Exp. Zool.*, LXXVIII, 1938.
- Etkin, W., "The Phenomena of Anuran Metamorphosis. III. The Development of the Thyroid Gland," *Jour. Morph.*, LIX, 1936.
- , and Huth, T., "A Thyrotropic Field Effect in the Tadpole. I," *Jour. Exp. Zool.*, LXXXII, 1939.
- Fcycleshymer, A. C., "The Development of Optic Vesicles in Amphibia," *Jour. Morph.*, VIII, 1893. — "The Early Development of *Amblystoma*, with Observations on some other Vertebrates," *Jour. Morph.*, X, 1895.
- Fales, D. E., "Experiments on the Development of the Pronephros of *Amblystoma punctatum*," *Jour. Exp. Zool.*, LXXII, 1935.
- Field, H. H., "The Development of the Pronephros and Segmental Duct in Amphibia," *B. M. C. Z. Harvard*, XXI, 1891. — "Die Vornierenkapsel, ventrale Musculatur und Extremitätenanlagen bei den Amphibien," *Anat. Anz.*, IX, 1894. — "Bemerkungen über die Entwicklung der Wirbelsäule bei den Amphibien," *Morph. Jahrb.*, XXII, 1895.
- Figge, F. H. J. and Uhlenhuth, E., "The Morphology and Physiology of the Salamander Thyroid Gland. VIII. Further Experiments on the Thyreo-Activator," *Physiol. Zool.*, VI, 1933.
- Gaupp, E., *Ecker und Wiedersheims Anatomie des Frosches*, Braunschweig, 1896, 1904. — "Ontogenese und Phylogenese des schalleitenden Apparates bei den Wirbeltieren," *Ergeb. Anat. u. Entw.*, VIII, 1899 (1898).
- Geinitz, B., "Zur Weiteren Analyse des Organisationszentrums," *Zeit. Ind. Abs. u. Vererb.*, XXXVII, 1925. — "Embryonale Transplantation zwischen Urodelen und Anuren," *Arch. Entw.-mech.*, CVI, 1925.
- Goerttler, K., "Die Formbildung der Medullaranlage bei Urodelen," *Arch. Entw.-mech.*, CVI, 1925.
- Goss, C. M., "Experimental Removal of the Blood Island of *Amblystoma punctatum* Embryos," *Jour. Exp. Zool.*, LII, 1928.
- Gudernatsch, J. F., "Feeding Experiments on Tadpoles. II. A Further Contribution to the Knowledge of Organs of Internal Secretion," *Am. Jour. Anat.*, XV, 1914.
- Hall, R. W., "The Development of the Mesonephros and the Müllerian Ducts in Amphibia," *B. M. C. Z. Harvard*, XLV, 1904.
- Harrison, R. G., "On Relations of Symmetry in Transplanted Limbs," *Jour. Exp. Zool.*, XXXII, 1921.
- Hegre, E. S., "The Developmental Relationship between the Nervous and Epithelial Components of the Hypophysis," *Jour. Exp. Zool.*, CI, 1946. — "The Developmental Stage at which the Intermediate Lobe of the Hypophysis Becomes Determined," *Jour. Exp. Zool.*, CIII, 1946.
- Held, H., *Entwicklung des Nervengewebe bei den Wirbeltiere*, Leipzig, 1909.
- Helff, O. M., "Studies on Amphibian Metamorphosis. I. Formation of the Opercular Leg Perforation in Anuran Larvæ during Metamorphosis," *Jour. Exp. Zool.*, XLV, 1926. — "Studies on Amphibian Metamorphosis. III. The Influ-

- ence of the Annular Tympanic Cartilage on the Formation of the Tympanic Membrane," *Physiol. Zööl.*, I, 1928. — "V. The Atrophy of Anuran Tail Muscle during Metamorphosis," *Physiol. Zööl.*, II, 1929. — "VIII. The Role of the Urostyle in the Atrophy of the Tail," *Anat. Rec.*, XLVII, 1930. — "VII. The Influence of the Columella on the Formation of the Lamina Propria of the Tympanic Membrane," *Jour. Exp. Zööl.*, LIX, 1931. — "XII. Potential Influences of the Quadratus and Supra-Scapula on Tympanic Membrane Formation in the Anuran," *Jour. Exp. Zööl.*, LXVII, 1934.
- Hempstead, M., "Development of the Lungs in the Frogs, *Rana catesbeiana*, *R. sylvatica* and *R. virescens*," *Science*, XII, 1901.
- Hertwig, O., "Experimentelle Untersuchungen über die ersten Theilungen des Froscheies und ihre Beziehungen zu der Organbildung des Embryo," *Sitzber. Ber. Akad.*, 1893. — "Ueber den Werth der ersten Furchungszellen für die Organbildung des Embryo Experimentelle Studien am Frosch- und Tritonei," *Arch. Mikr. Anat.*, XLII, 1893. (Editor), *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere*, Jena, 1906 (1901-1906).
- Holtfreter, J., "Morphologische Beeinflussung von Urodelenektoderm bei xenoplastischer Transplantation," *Arch. f. Entw.-mech.*, CXXX, 1935. — "A Study of the Mechanics of Gastrulation: Part I," *Jour. Exp. Zööl.*, LXLIV, 1943.
- Humphrey, R. R., "The Early Position of the Primordial Germ Cells in Urodeles; Evidence from Experimental Studies," *Anat. Rec.*, XLII, 1929. — "Studies on Sex Reversal in Amblystoma. VII. Reversal of Sex Type in Gonadic Preprimordia of *A. punctatum* males implanted in Females of More Rapidly Growing Species," *Anat. Rec.*, LXII, 1935. — "IX. Reversal of Ovaries to Testes in Parabioc *A. tigrinum*," *Jour. Exp. Zööl.*, LXXIII, 1936.
- Janes, R. G., "Studies on the Amphibian Digestive System. III. The Origin and Development of Pancreatic Islands in Certain Species of Anura," *Jour. Morph.*, LXII, 1938.
- Jenkinson, J. W., "On the Relation between the Symmetry of the Egg and the Symmetry of Segmentation and the Symmetry of the Embryo in the Frog," *Biometrika*, VII, 1909. — *Experimental Embryology*, Oxford, 1909.
- Kaan, H. W., "Further Studies on the Auditory Vesicle and Cartilaginous Capsule of *Amblystoma punctatum*," *Jour. Exp. Zööl.*, LXXXVIII, 1938.
- Knouff, R. A., "The Origin of the Cranial Ganglia of *Rana*," *Jour. Comp. Neur.*, XLIV, 1927. — "The Developmental Pattern of Ectodermal Placodes in *Rana pipiens*," LXII, 1935.
- Lehmann, F. E., "Further Studies on the Morphogenetic Role of the Somites in the Development of the Nervous System of the Amphibians. The Differentiation and Arrangement of the Spinal Ganglia in *Pleurodeles waltli*," *Jour. Exp. Zööl.*, XLIX, 1927.
- Lewis, W. H., "Experimental Studies on the Development of the Eye in Amphibians. I. On the Origin of the Lens," *Am. Jour. Anat.*, III, 1904.
- Liedke, K. B., "Lens Competence in *Rana pipiens*," *Jour. Exp. Zööl.*, LXL, 1942.
- Lindeman, V. F., "Integumentary Pigmentation in the Frog (*R. pipiens*) during Metamorphosis, with Special Reference to Tail Skin Histolysis," *Physiol. Zööl.*, II, 1929.
- Mangold, O., "Transplantationsversuche zur Frage der Spezifität unter der Bildung der Keimblätter," *Arch. Mikr. Anat.*, C, 1924.
- Marx, A., "Experimentelle Untersuchungen zur Frage der Determination der Medullarplatte," *Arch. Mikr. Anat.*, CV, 1925.
- Maximow and Bloom, *Text-Book of Histology*, Philadelphia, 1938.
- McClendon, J. F., "The Development of Isolated Blastomeres of the Frog's Egg," *Am. Jour. Anat.*, X, 1910.

258 THE FROG: LATER OR LARVAL DEVELOPMENT

- Morgan, T. H., "The Formation of the Embryo of the Frog," *Anat. Anz.*, IX, 1894. — "Half-embryos and Whole-embryos from One of the First Two Blastomeres of the Frog's Egg," *Anat. Anz.*, X, 1895. — *The Development of the Frog's Egg: An Introduction to Experimental Embryology*, New York, 1897. — "The Relation between Normal and Abnormal Development of the Embryo of the Frog, as Determined by Injury to the Yolk-Portion of the Egg," *Arch. Entw.-mech.*, XV, 1902. — "The Relation between Normal and Abnormal Development of the Embryo of the Frog (III), as Determined by Some Abnormal Forms of Development," *Arch. Entw.-mech.*, XVIII, 1904. — "The Relation between Normal and Abnormal Development of the Embryo of the Frog: X. A Re-examination of the Early Stages of Normal Development from the Point of View of the Results of Abnormal Development," *Arch. Entw.-mech.*, XIX, 1905. — "Experiments with Frog's Eggs," *Biol. Bull.*, XI, 1906. — "The Origin of the Organ-forming Materials in the Frog's Embryo," *Biol. Bull.*, XI, 1906. — *Experimental Embryology*, New York, 1928.
- Needham, Joseph, *Biochemistry and Morphogenesis*, Cambridge, 1942.
- Pasteels, J., "New Observations Concerning the Maps of Presumptive Areas of the Young Amphibian Gastrula (Amblystoma and Discoglossus)," *Jour. Exp. Zool.*, LXXXIX, 1942. — "On the Formation of the Primary Entoderm of the Duck (*Anas domestica*) and on the Significance of the Bilaminar Embryo in Birds," *Anat. Rec.*, LXLIII, 1945.
- Piatt, J., "Nerve-Muscle Specificity in Amblystoma, Studies by Means of Heterotopic Cord Grafts," *Jour. Exp. Zool.*, LXXXV, 1940.
- Porter, K. R., "Androgenetic Development of the Egg of *Rana pipiens*," *Biol. Bull.*, LXXVII, 1939.
- Raven, Chr. P., "Zur Entwicklung der Ganglienleiste. V. Über die Differenzierung des Rumpfganglienleistenmaterials," *Arch. Entw.-mech.*, CXXXIV, 1936.
- Roux, W., "Beiträge zur Entwicklungsmechanik des Embryos, Nr. IV. Die Richtungsbestimmung der Medianebene des Froschembryo durch die Copulationsrichtung des Eikernes und des Spermakernes," *Arch. Mikr. Anat.*, XXIX, 1887. — "Beiträge zur Entwicklungsmechanik des Embryo." V. "Ueber die künstliche Hervorbringung halber Embryonen durch Zerstörung einer der beiden ersten Furchungskugeln, sowie über die Nachentwicklung der fehlenden körperlälfte," *Virchow's Archiv.*, CXIV, 1888. — "Ueber die Lagerung des Materials des Medullarrohres im gefurchten Froschei (Verh. Anat. Ges. 2)," *Anat. Anz.*, III, 1888. — "Ueber die ersten Teilungen des Froscheies und ihre Beziehungen zu der Organbildung des Embryo," *Anat. Anz.*, VIII, 1893.
- Rugh, Roberts, "Heterchromatic Radiations and Early Amphibian Development," *Coll. Net.*, VIII, 1933. — "A Quantitative Analysis of the Pituitary-Ovulation Relation in the Frog (*Rana pipiens*)," *Physiol. Zool.*, X, 1937. — "Release of Spermatozoa by Anterior Pituitary Treatment of the Male Frog, *Rana pipiens*," *Proc. Soc. Exp. Biol. and Med.*, XXXVI, 1937.
- Schechtman, A. M., "Unipolar Ingression in Triturus: A Hitherto Undescribed Movement in the Pregastrular Stages of a Urodele," *Univ. Cal. Press*, XXXIX, 1934. — "Mechanism of Ingression in the Egg of *Triturus torosus*," *Proc. Soc. Exp. Biol. and Med.*, XXXII, 1935. — "The Mechanism of Amphibian Gastrulation. I. Gastrulation-Promoting Interactions Between Various Regions of an Anuran Egg (*Hyla regilla*)," *Univ. Cal. Press*, LI, 1942.
- Schleip, W., *Die Determination der Primitiventwicklung*, Leipzig, 1929.
- , W. and Penners, A., "Weitere Untersuchungen über die Entstehung der Schultzeschen Doppelbildungen beim braunen Frosch," *Ver. Phys.-Med. Ges.*, Würzburg, LI, 1926.
- Schötté, O. E. and Edds, Mac V., "Xenoplastic Induction of *Rana pipiens* Ad-

- hesive Discs on Balancer Site of *Amblystoma punctatum*," *Jour. Exp. Zool.*, LXXXIV, 1940.
- Schultze, O., "Die kuenstliche Erzeugung von Doppelbildungen bei Froschlarven mit Hilfe abnormer Gravitationswirkung," *Arch. Entw.-mech.*, I, 1894.
- Schwind, J. L., "Tissue Specificity at the Time of Metamorphosis in Frog Larvae," *Jour. Exp. Zool.*, LXVI, 1933.
- Shore, T. W., "On the Development of the Renal-portals and Fate of the Posterior Cardinal Veins in the Frog," *Jour. Anat. Physiol.*, XXXVI, 1901.
- Smith, P. E., "The Pigmentary Growth and Endocrine Disturbances Produced in the Anuran Tadpole by the Ablation of Pars Bucalis of the Hypophysis," *Am. Anat. Mem.*, II, 1920.
- Spemann, H., "Entwicklungsphysiologische Studien am Triton-Ei," I, II, III, *Arch. Entw.-mech.*, XII, XV, XVI, 1901-1903. — "Ueber experimentelle erzeugte Doppelbildungen mit cyclopischem Defekt," *Zool. Jahrb. Supplement*, VII, 1904. — "Ueber die Determination der ersten Organanlagen des Amphibienembryo," I-II, *Arch. Entw.-mech.*, XLIII, 1918. — "Die Erzeugung tierischer Chimären durch heteroplastische embryonale Transplantation zwischen Triton cristatus und Triton taeniatus," *Arch. Entw.-mech.*, XLVIII, 1921. — "Ueber Organisatoren in der tierischen Entwicklung," *Nat.-Wiss.*, XII, 1924. — "Some Factors in Animal Development," (Translation), *Brit. Jour. Exp. Biol.*, II, 1925.
- , and Mangold, H., "Ueber Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren," *Arch. Mikr. Anat.*, C, 1924.
- Swett, F., "Experiments upon Delayed Determination of the Dorsoventral Limb Axis in *Amblystoma punctatum* (Linn.)," *Jour. Exp. Zool.*, LXXV, 1937. — "Further Experiments upon the Establishment and the Reversal of Prospective Dorsoventral Limb-Axis Polarity," *Jour. Exp. Zool.*, LXXXII, 1939. — "Establishment of Definitive Polarity in the Dorsoventral Axis of the Fore-limb Girdle in *Amblystoma punctatum* (Linn.)," *Jour. Exp. Zool.*, LXXXVI, 1941.
- Swingle, W. W., "The Germ Cells of Anurans," I. "The Male Sexual Cycle of *Rana catesbeiana*," II. "An Embryological Study of Sex Differentiation in *Rana catesbeiana*," *Jour. Morph. and Physiol.*, XLI, 1926.
- Taylor, A. C., "Selectivity of Nerve Fibers from the Dorsal and Ventral Roots in the Development of the Frog Limb," *Jour. Exp. Zool.*, LXLVI, 1944.
- , and Kollros, J. J., "Stages in the Development of *Rana pipiens* Larvae," *Anat. Rec.*, LXLIV, 1946.
- Vogt, W., "Operativ bewirkte 'Exogastrulation' bei Triton und ihre Bedeutung für die Theorie der Wirbeltiergastrulation," *Verh. d. Anat. Gesell.*, LV, 1922. — "Gestaltungsanalyse am Amphibienkeim mit örtlicher Vitalfärbung," *Arch. Entw.-mech.*, CVI, 1925. — "Ueber Wachstum und Gestaltungsbewegungen am hinteren Körperende der Amphibien," *Verh. d. Anat. Gesell.*, LXI, 1926.
- Weber, A., "Étude de la torsion de l'ébauche cardiaque chez *Rana esculenta*," *Bibliographie Anatomique*, XVIII, 1908 (1909).
- Wilder, H. H., *History of the Human Body*, New York, 1909.
- Witschi, E., "Studies on Sex Differentiation and Sex Determination in Amphibians," I. "Development and Sexual Differentiation of the Gonads of *Rana sylvatica*," *Jour. Exp. Zool.*, LII, 1929. — "Studies on Sex Differentiation and Sex Determination in Amphibians. II. Sex Reversal in Female Tadpoles of *Rana sylvatica* Following the Application of High Temperature," *Jour. Exp. Zool.*, LII, 1929. — "VI. The Nature of Bidder's Organ in the Toad," *Am. Jour. Anat.*, LII, 1933. — "VIII. Experiments on Inductive Inhibition of Sex Differentiation in Parabioc Twins of a Salamander," *Anat. Rec.*, LXVI, 1936. — "IX. Quantitative Relationships in the Induction of Sex Differentiation, and

260 THE FROG: LATER OR LARVAL DEVELOPMENT

- the Problem of Sex Reversal in Parabiatic Salamanders," *Jour. Exp. Zool.*, LXXV, 1937. — "The bronchial columella of the ear of larval Ranidae," *Jour. Morph.*, XCVI, 1955.
- Yntema, C. L., "An Experimental Study of the Origin of the Cells which Constitute the VIIth and VIIIth Cranial Ganglia and Nerves in the Embryo of *Amblystoma punctatum*," *Jour. Exp. Zool.*, LXXV, 1937. — "An Experimental Study on the Origin of the Sensory Neurones and Sheath Cells of the IXth and Xth Cranial Nerves in *Amblystoma punctatum*," *Jour. Exp. Zool.*, LXLII, 1943.
- Zwilling, E., "An Experimental Analysis of the Development of the Anuran Olfactory Organ," *Jour. Exp. Zool.*, LXXXIV, 1940. — "The Determination of the Otic Vesicle in *Rana pipiens*," *Jour. Exp. Zool.*, LXXXVI, 1941.

APPENDIX TO FROG BIBLIOGRAPHY

- Ballard, W. W., "Cortical ingression during cleavage of Amphibian eggs, studied by means of vital dyes," *Jour. Exp. Zool.*, CXXIX, 1955.
- Barth, L. G., *Embryology*, New York, 1953.
- Bruner, J. A. and Witschi, E., "Pluripotentiality of the mesonephric blastema and the mechanism of feminization of male salamanders by androgenic hormones," *Anat. Rec.*, CXX, 1954.
- Calkins, C. N., "The restoration of vitality through conjugation," *Proc. Natl. Acad. Sci.*, V, 1919.
- Chang, C. Y., "Parabiosis and gonad transplantation in *Xenopus laevis* daudin," *Jour. Exp. Zool.*, CXXIII, 1953.
- Finnegan, C. V., "Studies on erythropies in salamander embryos," *Jour. Exp. Zool.*, CXXIH, 1953.
- Hibbard, H., "Contributions à l'étude de l'Ovogenese de la fecondation, et de l'histogenèse chez *Discoglossus pictus* oth," *Arch. de Biol.*, XXXVIII, 1928.
- Holtzer, H., "An experimental analysis of the development of the spinal column. I. The dispensability of the notochord," *Jour. Exp. Zool.*, CXXI, 1952.
- Jennings, H. S., "Paramecium bursaria: Life History. Age and death of clones in relation to the results of conjugation," *Jour. Exp. Zool.*, XCVI, 1944.
- Kemp, N. E., "Synthesis of yolk in the oocytes of *Rana pipiens* after induced ovulation," *Jour. Morph.*, LXLII, 1953.
- Kollros, J. J., "The development of the optic lobes in the Frog. I. The effects of unilateral enucleation in embryonic stages," *Jour. Exp. Zool.*, CXXIII, 1953.
- Liedke, K. B., "Lens competence in *Amblystoma punctatum*," *Jour. Exp. Zool.*, CXVII, 1951.
- Nicholas, J. S., "Blastulation, its role in pregastrular organization in *Amblystoma punctatum*," *Jour. Exp. Zool.*, C, 1945.
- Nieuwkoop, P. D., "Experimental investigations on the origin and determination of the germ cells and on the development of the lateral plates and germ ridges in Urodeles," *Arch. Neerl. Zool.*, VIII, 1947.
- Segal, S. J., "Morphogenesis of the oestrogen induced hyperplasia of the adrenals in larval frogs," *Anat. Rec.*, CXV, 1953.
- Sonneborn, T. M., "Mating types and groups, lethal interactions; determination and inheritance," *Am. Nat.*, LXXIII, 1939.
- Ting, Han-po Paul, "Diploid androgenetic and gynogenetic haploid development in Anuran hybridization," *Jour. Exp. Zool.*, CXVI, 1951.
- Townes, P. L. and Holtfreter, J., "Directed movements and selective adhesion of embryonic Amphibian cells," *Jour. Exp. Zool.*, CXXIX, 1955.
- Wilens, S., "The migration of heart mesoderm and associated areas in *Amblystoma punctatum*," *Jour. Exp. Zool.*, CXXIX, 1955.
- Wittek, M., "La vitellogenese chez les Amphibiens," *Arch. de Biol.*, LXIII, 1952.
- Woodruff, L. A. and Erdmann, R., "A normal periodic reorganization process without cell fusion in *Paramecium*," *Jour. Exp. Zool.*, XVII, 1914.

PART III

THE TELEOSTS AND GYMNOPHIONA

T

HE TELEOSTS AND GYMNOPHIONA: THEIR SEG- MENTATION AND GASTRULATION

BEFORE beginning the study of the Chick, it is desirable to give a very brief account of the processes of segmentation and gastrulation in the Teleosts (Bony Fishes) and the Gymnophiona. It is of advantage to understand these processes in the forms mentioned because embryologically they are intermediate between those found in the Frog and those in the Reptile or Bird, i.e., the Sauropsids. This of course is not meant to imply that *modern* Fishes, Amphibians, and Sauropsids form a direct phylogenetic series. It is merely suggestive in a general way of the manner in which the embryological conditions in the lower forms have apparently been modified in the process of evolution.

THE TELEOSTS

SEGMENTATION

In the Frog the yolk is more or less concentrated in the vegetal half of the egg, but is not sufficiently dense to prevent the whole egg from segmenting. In the Teleosts, on the contrary, the concentration of yolk is very marked, so that the protoplasm exists only as a thin plate upon the animal pole. As noted in Chapter II, this plate is called the *blastodisc*, and from it the entire embryo arises, the remainder of the egg being purely nutritive. In these eggs, therefore, when segmentation begins, the process is confined to this disc, and is said to be *meroblastic* or *discoidal*, as opposed to the holoblastic or total cleavage of *Amphioxus* and the Frog (Fig. 136).

The first two planes of division pass entirely through the disc and at right angles to one another. Normally the third cleavage is at right angles to the second, so that at this point the pattern may be described as bilateral with respect to the plane of the latter cleavage. This feature is further emphasized in many Teleost eggs by the temporary lengthening of the blastodisc along the axis of this second plane. Thus instead of being circular at this stage the disc is an oval (almost an oblong), its long axis commonly consisting of two rows of four cells each. The

fourth cleavages then generally come in at right angles to the first so that we have four rows of four cells each, two on either side of the second cleavage plane, i.e., on either side of the long axis of the oval (Fig. 137, C). However, shortly after this the dividing blastodisc ceases to be

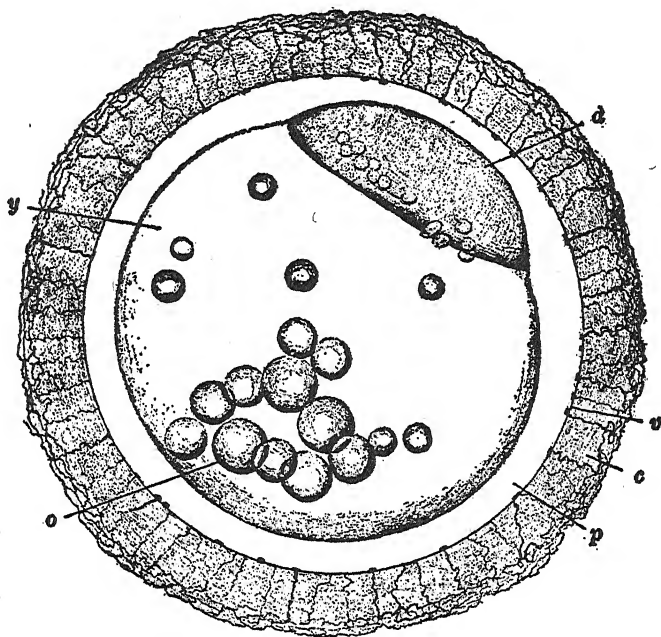


Fig. 136. — Egg of the Teleost, *Fundulus heteroclitus*. From Kellicott (*General Embryology*). Total view, about an hour after fertilization.

c. Chorion. d. Protoplasmic germ disc or blastodisc. o. Oil vacuoles. p. Perivitelline space. v. Vitelline membrane. y. Yolk.

an oval and again becomes circular. Further cleavages ensue, and the disc is thus presently transformed into the *blastoderm*. This consists of small cells whose original relationships are impossible to trace unless vital stains or other means of identification have been employed. Between this blastoderm and the yolk, a space has meanwhile developed, which is termed the *segmentation cavity*, and which corresponds to the cavity of the same name in the Frog (Fig. 137, D, E). Thus the egg has become a *blastula*.

In the yolk around the margin of the blastoderm are a number of nuclei (*yolk nuclei*) derived partly from the blastodermal edge, and partly perhaps from extra sperm (merocytes). These nuclei presently begin to divide amitotically, and become amoeboid, in some cases mi-

grating centrally beneath the blastoderm. Here they occupy the thin layer of protoplasm forming the floor of the segmentation cavity, which thus has the character of a syncytium. This syncytium or *periblast*, as it is termed, presently spreads over the entire yolk, and is perhaps instrumental in making the latter assimilable by the cells of the blastoderm. At all events, it finally disappears without taking any part in the formation of the actual embryo; hence it need not be considered further.

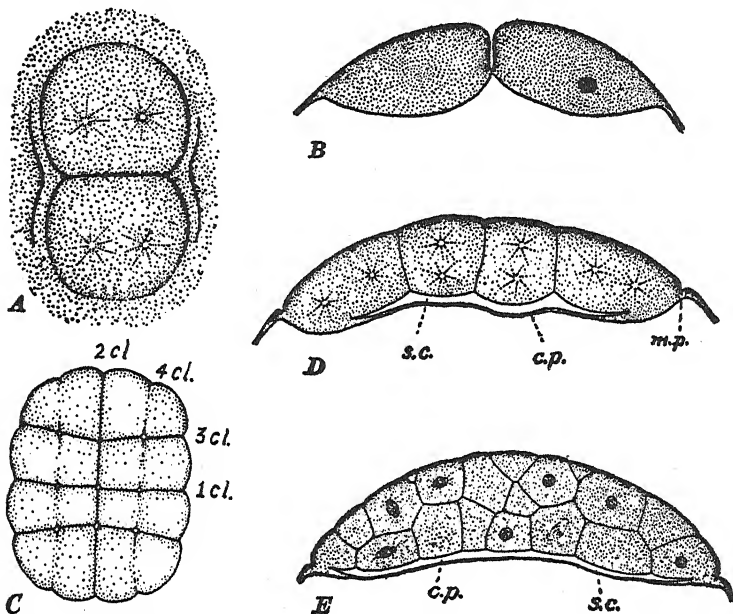


Fig. 137. — Cleavage in the Sea-bass, *Serranus atrarius*. From H. V. Wilson. A. Surface view of blastoderm in two-cell stage. B. Vertical section through four-cell stage. C. Surface view of blastoderm of sixteen cells. D. Vertical section through sixteen-cell stage. E. Vertical section through late cleavage stage.

c.p. Central periblast. m.p. Marginal periblast. s.c. Segmentation cavity (blastocoel).

GASTRULATION

There have been several attempts to discover what determines the antero-posterior axis in the Fish, but none in the writer's opinion has been very successful, including his own. It is a fact that in the forms which have been studied this axis usually coincides with the second plane of cleavage. But this is not always so, and what causes the variation no one really knows. Whatever the determining factor or factors may be the axis becomes manifest with the advent of gastrulation.

Involution.— In that region of the blastoderm which is destined to form the posterior end of the animal, the blastodermal rim begins to turn under, i.e., is involuted. Thus, in this region a lower layer of cells begins to spread anteriorly into the segmentation cavity beneath the blastoderm. It is the *hypoblast*, destined later to give rise to the endoderm, notochord and mesoderm, while the remaining upper layer is the *epiblast*. The margin of the blastoderm where the involution is occur-

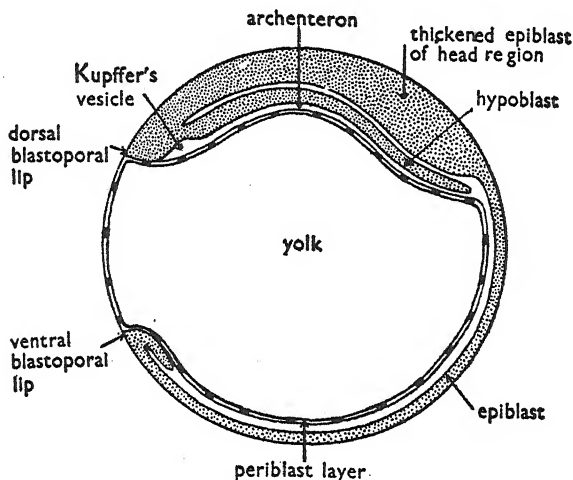


Fig. 138.— Diagram of a median sagittal section of a Teleost gastrula shortly before the closure of the blastopore. From H. V. Wilson, slightly modified.

ring, constitutes the dorsal blastoporal lip, while the former segmentation cavity now lying between the spreading hypoblast and the yolk is the *archenteron* (Figs. 138, 139). The new cavity, like its predecessor, is obviously extremely shallow, and though roofed by the hypoblast is open below to the surface of the yolk, or more strictly speaking to the thin syncytial layer of periblast. Lastly, it is to be noted that while the process of involution is most active at the posterior edge of the blastoderm, it is also occurring to a much lesser degree all around the margin.

Epiboly.— While involution is thus progressing chiefly at the posterior edge of the blastoderm, very active epiboly is taking place about the remainder of the blastodermal margin, the result being to envelop the entire yolk with an epiblastic covering of cells, the *yolk-sac*, and concurrently to close the blastopore. In such cases, as suggested in Chapter II, it is possible to regard the entire rim of the growing blastoderm as the *blastoporal lip*. Thus while the posterior edge is recognized

as the dorsal lip, the lateral edges must be regarded as the lateral lips and the anterior edge as the ventral lip. It may be noted, furthermore, that in some forms, e.g., *Serranus*, the Sea Bass, according to Wilson ('89), the epibolic process is most rapid at the anterior edge (ventral lip), and decreases along either side until at the posterior edge (dorsal lip) it is comparatively slight. Under such circumstances the above homology is particularly obvious because, owing to its relatively rapid growth, the anterior edge passes clear around the vegetal pole and up

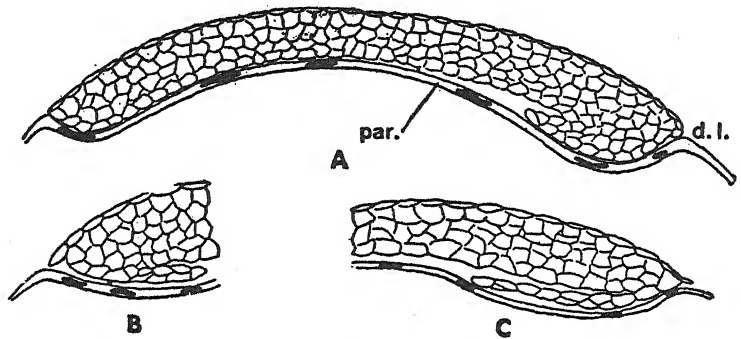


Fig. 139. — Sagittal sections through the blastoderm of *Serranus* during the formation of the germinal layers. From Jenkinson (*Vertebrate Embryology*). After H. V. Wilson. A. Beginning of involution and slight epiboly at dorsal lip (d.l.). B. Epiboly at anterior edge. C. Further progress of involution at dorsal lip. d.l. Dorsal lip. par. Parablast (periblast).

on to the posterior side, thus becoming actually a ventral lip in position as well as in name (Figs. 138, 140). How widespread among Fish eggs this characteristic of the relatively excessive growth of the anterior edge of the blastoderm may be cannot be definitely stated, because in most descriptions the point is not made clear. This is due partly perhaps to difficulty in many cases of being sure of the constant orientation of the parts of the egg, which in the Sea Bass is said to be fixed by the position of the oil globule. In at least one other instance, however, i.e., that of the oval egg of *Hemichromis* (McEwen '30), this orientation is equally well or better established by the shape of the egg. In this case the blastoderm is at one end of the oval, and the egg does not normally turn end over end within its chorionic membrane because of the stiffness of the latter and its own viscosity. It is thus possible to observe that epiboly, unlike that in *Serranus*, is clearly equal on all sides. Hence the blastopore obviously closes on exactly the opposite side (end) of the egg from the original animal pole (Fig. 141).

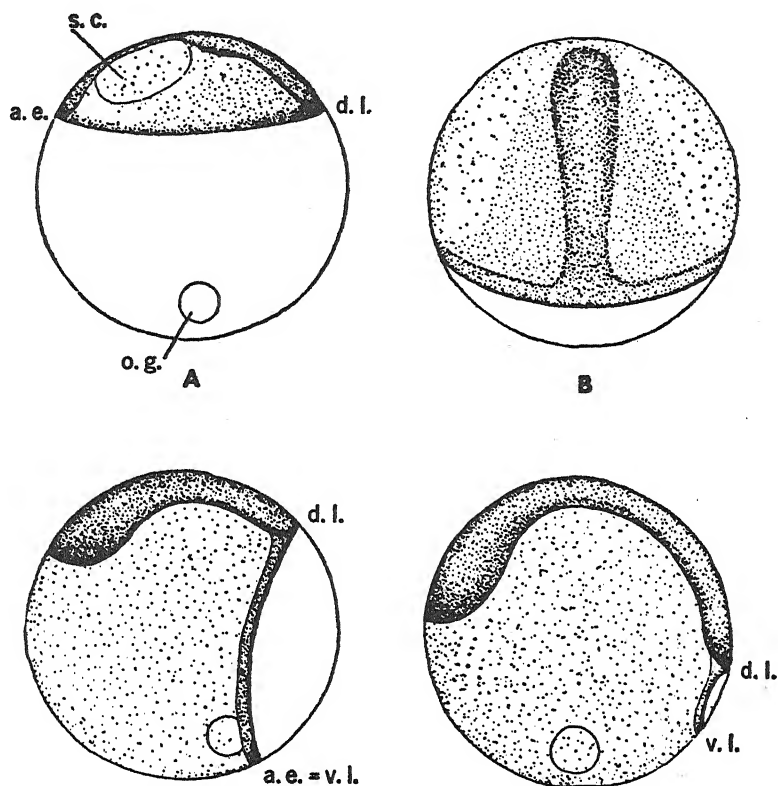


Fig. 140. — Growth of the blastoderm over the yolk (epiboly) after the formation of the material for the embryo in the Teleostan fish *Serranus*. From Jenkinson (*Vertebrate Embryology*). After H. V. Wilson. A Lateral view of a very early stage of gastrulation. B. Dorsal view of a much later stage. C. Lateral view of the same stage as B. D. Lateral view of a late stage, gastrulation almost complete.

d.l. Dorsal lip of the blastopore (posterior edge of the blastoderm). *a.e.* Anterior edge of the blastoderm or ventral lip (*v.l.*) of the blastopore. *s.c.* Segmentation cavity. *o.g.* Oil globule.

Concrescence or Convergence. — The Fish, as previously stated, is the form in connection with which the theory of concrescence originated, and it may be that this process does occur here to a limited extent. However, as in other cases, it is now considered that the movement which takes place in this form is more aptly designated as convergence (Oppenheimer, '36). It goes on of course along with the epiboly, and seems to involve two things. There is on the one hand some actual concrescence or confluence of material in the germ ring on either side of the dorsal lip of the blastopore. The greater part of this material, how-

ever, flows more directly, partly toward the lip and partly toward the median line, i.e., it converges toward these regions (Fig. 142). This and the involution leads to a piling up of cells in a somewhat shield shaped area anterior to the dorsal blastoporal lip, the base of the shield being

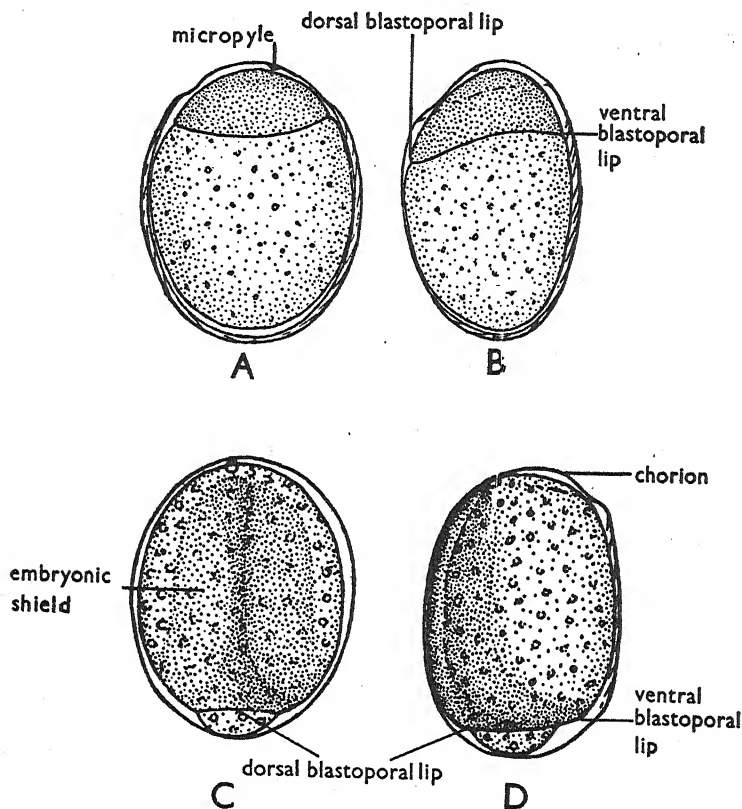


Fig. 141. — *A* and *B* early stages, *C* and *D*, late stages in the gastrulation of the Teleost, *Hemichromis bimaculata*. *A* and *C* are dorsal views, *B* and *D* are lateral views. Note the equal epibolic growth of the blastoporal lips, unlike the condition in *Serranus*.

adjacent to the lip. This area is in fact known as the *embryonic shield*, and it is along its median longitudinal axis that the outline of the embryo presently appears as indicated in Fig. 141, *C*.

Meanwhile as the lips of the blastopore finally close posterior to the shield they leave, at least in some embryos (Sea Bass, H. V. Wilson), a short thickened line of cells. At the anterior end of this line is a slight cavity extending upward from the shallow archenteron (Figs. 138, 143).

It is called *Kupffer's vesicle*, and seems to be an incipient neurenteric canal. It cannot be a genuine neurenteric canal since the nerve cord, because of its peculiar method of formation in the Fish, does not yet itself possess a lumen. At the posterior end of the thickened line is the place of final blastoporal closure, and probably also the place where the future anus opens. However, since the Fish unlike the Frog does not have

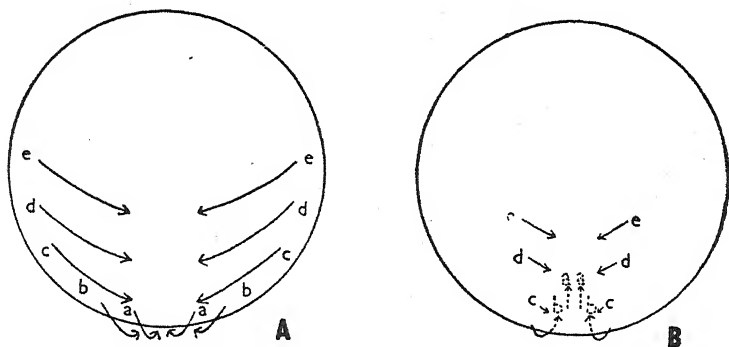


Fig. 142. — A diagrammatic representation of the process of convergence, and incidentally a small amount of involution, essentially as they are thought to occur in the Teleosts, as well as in some other forms. *A*. Surface view of the blastoderm at the beginning of the processes. *B*. A similar view near the completion of gastrulation. Changes in the positions of the letters and the directions of the arrows represent the movements which are supposed to have occurred. Dotted letters and arrows indicate regions which have been involuted underneath the margin, and hence would be invisible from above.

a proctodaeal invagination to mark this spot, the latter point is not certain. Assuming, nevertheless, the homology of Kupffer's vesicle with a neurenteric canal, and the place of blastoporal closure with the anus, the thickened line is evidently the homologue of the *primitive streak* of the Amphibian. On this basis it may be so designated. The mass of cells in and around the more posterior portion of it, however, because of their character and future history, are often designated as the *caudal knob*. Thus is produced the Teleostean *gastrula*.

THE DIFFERENTIATION OF MESODERM, NOTOCHORD, AND DEFINITIVE ENDODERM

It has been stated that involution occurred chiefly at the dorsal lip of the blastopore. The result is that in the region anterior to this lip, i.e., the region of the embryonic shield, the roof of the archenteron consists of an extensive double layer of cells produced by this process. From the dorsal side of the lower or involuted of these two layers (hypoblast),

between it and the overlying epiblast, the *mesoderm* is now delaminated in two sheets situated upon either side of the middle line (Fig. 144). Presently, also, the hypoblast along the middle line itself becomes separated from that upon either hand, and is aggregated into an axial rod, the *notochord*, with the sheets of mesoderm upon each side of it (Figs. 144, 145). What remains of the original hypoblast may now be spoken of as *endoderm*, destined to form the lining of the gut. Since, however,

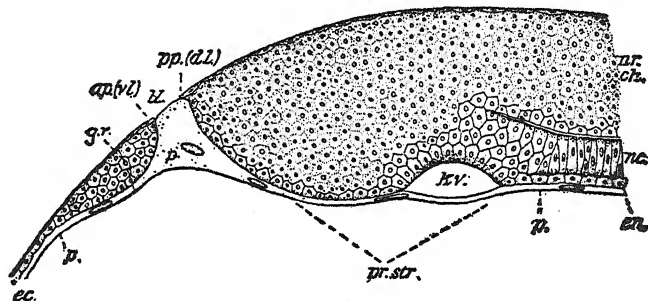


Fig. 143. — Sagittal section through the hinder end of a Fish embryo (*Serranus*), showing the undifferentiated primitive streak, anterior to which the structures of the embryo are being differentiated. From H. V. Wilson.

a.p. (v.l.). Anterior margin of the blastoderm or ventral lip of blastopore, after having grown entirely around the yolk mass. *bl.* Blastopore. *ec.* Ectoderm. *en.* Endoderm. *g.r.* Germ ring. *k.v.* Kupfer's vesicle. *nc.* Notochord. *nr. ch.* Nerve cord. *p.* Periblast. *pp. (d.l.)*. Posterior margin of blastoderm (dorsal lip of blastopore). *pr. str.* Primitive streak.

the formation of the notochord consumed all of the primordial cells along its line of origin, the definitive endoderm consists for a short time of two separate lateral sheets. Shortly, these sheets unite with one another beneath the notochord, and the enteric roof is thus again complete. The uppermost layer may now of course be designated as *ectoderm*.

CONSIDERATIONS CONCERNING THE ULTIMATE ORIGINS OF THESE LAYERS

It remains to be noted that although the involution of the hypoblast comprising potential endoderm, mesoderm and notochord, occurs chiefly at the dorsal blastoporal lip, the material for these layers does not all originate here. As in the case of the Frog we have seen that about this region there exists a process of convergence whereby materials anterior and lateral to the lip are carried toward it before they are involuted to the interior. The pregastrular locations of the different components of this hypoblast are indicated somewhat diagrammatically in Figure 146

taken from Oppenheimer's studies on *Fundulus*. Her conclusions were reached both by various grafting experiments and, as in the Amphibia, by marking with vital stains. From them it appears that at least a considerable part of the mesoderm and endoderm of the Fish embryo is derived from the posterior third or so of the blastoderm and from its margins.

Oppenheimer has also confirmed earlier work of a different sort by Stockard to the effect that the more anterior parts of the blastoporal lip have capacities which are not normally realized. Thus any part of the blastodermal margin if cut out and implanted into the embryonic shield may differentiate into a variety of structures which it would never form in its usual location. This may suggest an inductive effect on the transplanted material by the substance of the shield. It also may

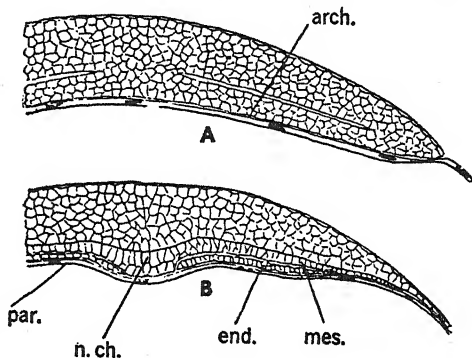


Fig. 144. — Transverse sections through the differentiating blastoderm of *Serranus* showing differentiation of the roof of the archenteron into notochord (n.ch.), mesoderm (mes.), and endoderm (end.). par. Parablast (periblast). From Jenkinson (*Vertebrate Embryology*). After H. V. Wilson.

mean that the material in various parts of the margin possesses inherent potentialities which are normally inhibited as this material is involuted over the dorsal blastoporal lip (Oppenheimer, '38). To this limited extent therefore the blastodermal margin (entire lip of the blastopore) may still be thought of as containing potentially the germ of any part, or all, of an embryo. Hence in this highly modified sense the use of the term germ ring as applied to this margin may still be justified. Finally, in connection with matters pertaining to pregastrular materials, Oppenheimer ('36) finds that blastoderms removed from the yolk and periblast previous to the 16-cell stage fail to gastrulate. Instead they behave somewhat like the upper quartet of cells from a Triton 8-cell stage which have been isolated from the lower four cells containing the gray crescent. For this reason this investigator suggests that perhaps the periblast of the 16-cell *Fundulus* contains a substance which influences the later destinies of these cells, but which has not previously had time to act. There is thus the implication that perhaps this periblastic substance has

an organizing effect somewhat comparable to that which occurs in the gray crescent region of the Amphibian.

EARLY FORMATION OF THE EMBRYO

As soon as the germ layers are formed in the embryonic region of the blastoderm, and while the remainder of the latter is still in the process of enclosing the yolk, the outlines of the embryo begin to become

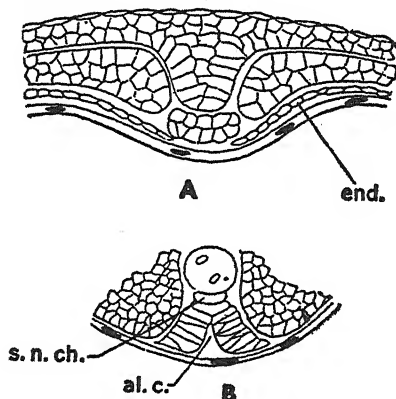


Fig. 145. — Formation of the gut (*al.c.*) in *Serranus* by the bending down of the sides of the roof of the archenteron. In *A* note also the nerve cord forming by a solid invagination of ectoderm (characteristic of many Teleosts) instead of by folds. From Jenkinson (*Vertebrate Embryology*). After H. V. Wilson.

s.n.ch. Sub-notochordal rod. *end.* Endoderm.

clearly evident. This is the result of a folding off process by which the embryo is gradually raised above the surface of the yolk. It is to be noted that although these procedures are fundamentally similar to what will presently be described in the Bird, there is one important difference. In the latter, in spite of the constriction of all three layers beneath the embryo due to the folding off, all three nevertheless take part in enclosing the yolk mass. In the Fish on the other hand the folding off of the endoderm is quickly completed to form a closed tube, the primitive gut. Hence only the ectoderm and mesoderm constitute the rather wide yolk stalk, and the covering

of the yolk, the *yolk-sac* (Figs. 144, 147). Aside from this difference further early development of Fish and Bird is generally similar. By virtue of the folding, accompanied by rapid growth in all directions, the embryo soon comes to extend outward above the yolk-sac which is attached like a bag to its ventral side. The tail in the Fish, it may be noted, is largely formed by outgrowth from the caudal knob.

THE GYMNOPTIONA

SEGMENTATION

Segmentation in these somewhat aberrant Amphibians is again virtually meroblastic, and hence results in the formation of a *blastula* with

a *blastoderm* and *segmentation cavity* very similar to that of the Teleost. In this case, it is true, there is a slight superficial cleavage in the yolk which forms the floor of the cavity, and this also extends out around the periphery of the blastoderm. The bulk of the yolk nevertheless, remains undivided.

GASTRULATION

Involution and Epiboly.—The advent of gastrulation becomes evident by the occurrence of involution and epiboly at what proves to be the posterior edge of the blastoderm, i.e., the dorsal blastoporal lip. As an obvious result of the involution there are presently produced the usual two layers of cells. The outer is the *epiblast* beneath which the inner *hypoblast* spreads out within the segmentation cavity above the partially segmented yolk. The method is made evident by reference to the median longitudinal sections of the blastoderm in Figure 148, *A* and *B*. Up to this point, it will be noted, the movements observed are not essentially different from those which took place at a corresponding stage in the Fish. The point in which the gastrulation of the Gymnophiona digresses from that in the forms thus far studied and to a slightly greater degree resembles that in the Birds, remains, therefore, to be noted.

The Gymnophionian Blastopore.—A surface view of the blastoderm as gastrulation commences (Fig. 149, *A*), will reveal the fact that the posterior portion of the rim where involution is occurring has the shape of a wide crescent, whose ends or horns bend backward. As the process goes on, moreover, these horns continue to grow posteriorly, and presently turn toward one another until they meet (Fig. 149, *B*, *C*, *D*). It is furthermore to be noted that this movement has occurred rela-

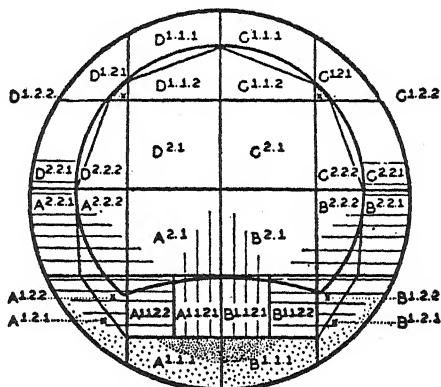


Fig. 146. — A diagram of an early Teleost (*Fundulus*) blastula. After Oppenheimer. The cells have been numbered for identification purposes in discussion of subsequent stages by the author, but are not pertinent to the account in this text. The point to be noted here is the location at this stage of the areas which will later form nervous system (vertical hatching), notochord (heavy stipple), endoderm (light stipple) and mesoderm (horizontal hatching).

tively rapidly, whereas the epiboly of the anterior side of the blastoderm, so rapid in the Fish, has scarcely started. The results of these processes compared with those in the Teleosts, as well as with those in forms with less yolk, may now be stated as follows:

If the entire blastodermal rim is still regarded as the *lip of the blasto-*

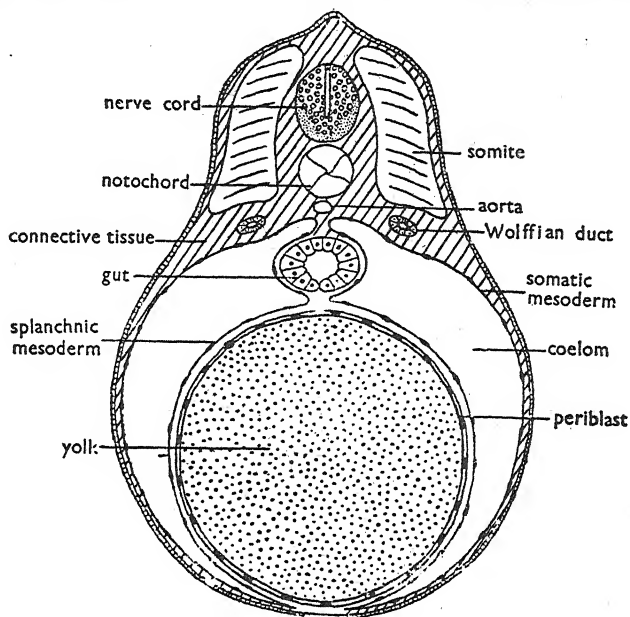


Fig. 147. — A diagram to illustrate the method of gut formation and yolk coverage in the Fish. Note that the endoderm has folded in to form the gut without covering the yolk at all, i.e., there is no endoderm in the yolk-sac. The latter is covered by the periblast (not a permanent cell layer) and by the two layers of mesoderm and the ectoderm. The extent of the coelom at this stage is exaggerated in the diagram.

pore (germ ring), it must be said that the movements just noted have divided this lip into two portions. One of these is quite limited; i.e., it merely furnishes the boundary for the small area of yolk (*yolk plug*) enclosed by the fused horns of the crescent (Fig. 149, C). The second portion of the original lip, on the other hand, bounds the entire remaining expanse of uncovered yolk. Moreover, since epiboly has been slight, this expanse comprises almost as much yolk surface as existed prior to the beginning of gastrulation. Such is the situation thus far indicated. Upon the basis of subsequent development, however, it may be stated

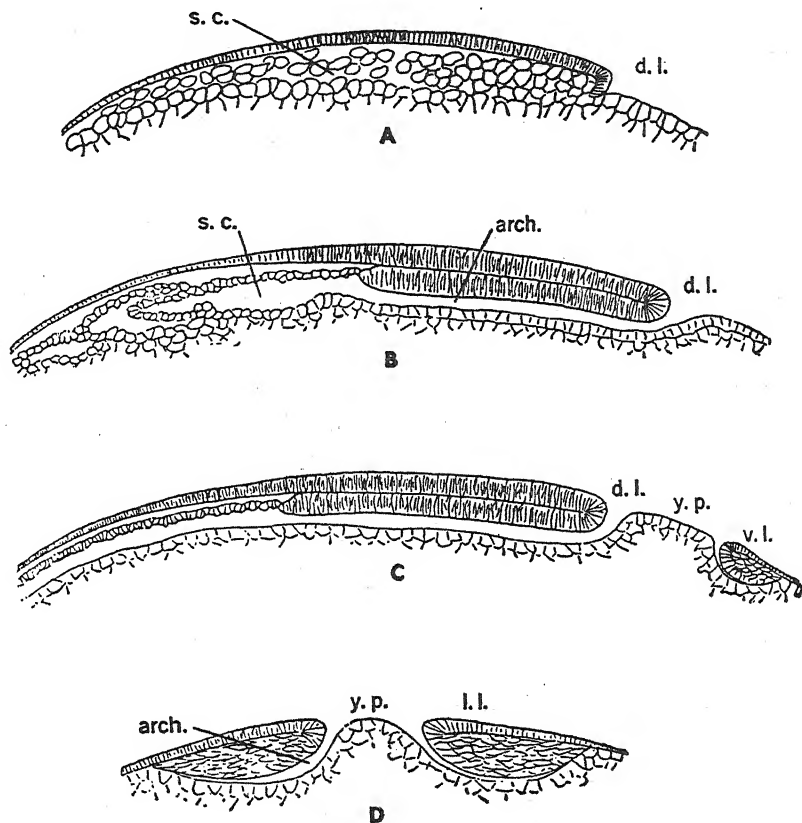


Fig. 148. — Formation of the germ layers in *Hypogeophis* (a Gymnophionian). From Jenkinson (*Vertebrate Embryology*). After Brauer. A–C. Sagittal sections of three successive stages. D. Transverse section through the blastopore and yolk plug (y.p.).

s.c. Segmentation cavity into which in B and C the archenteron (arch.) opens. d.l. Dorsal lip. l.l. Lateral-lip. v.l. Ventral lip.

that the small area enclosed by the horns of the crescent is the only part which really corresponds to the blastopore in the forms previously studied. Hence, as would be expected, its ultimate closure gives rise to a line of tissue quite homologous with the typical *primitive streak*, the *neurenteric canal* arising at its anterior end and the *anus* at the other. From this it appears that in the Gymnophiona, the anterior and most of the lateral parts of the blastodermal rim take no part in forming the ventral and lateral lips of the region which must be homologized with

a true blastopore, these lips being formed by the horns of the crescent. Instead, the outer (anterior and most of the lateral) portions of the rim are occupied merely with the gradual covering of the main body of the yolk, long after the true blastopore has been definitely delimited. Whether any convergence takes place before or during this delimitation has not been ascertained. Very possibly it does.

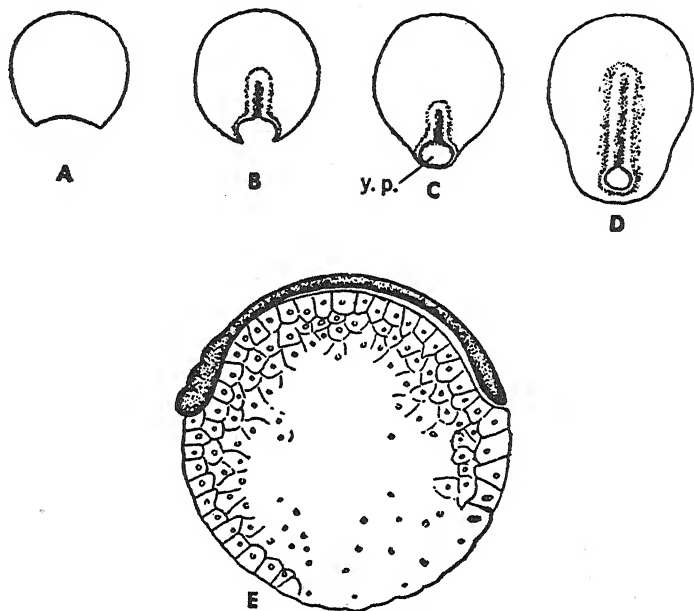


Fig. 149.—Formation and closure of the blastopore in the Gymnophiona. From Jenkinson (*Vertebrate Embryology*). A–D. Surface views of the blastoderm of *Hypogeophis*. After Brauer. The lateral lips are seen to meet behind and so form the ventral lip. y.p. Yolk plug. E. Embryo of *Ichthyophis* lying on the partially segmented yolk which is still uncovered by the blastoderm. After the brothers Sarasin.

It may now be noted that it is with respect to the relation of gastrulation proper and the belated enclosure of the yolk that the Gymnophiona come a step nearer to the condition in the Bird. In the latter also, as we shall see, gastrulation, so far as the embryo is concerned, is completed long before the mass of the yolk is covered by the epiboly of the blastodermal rim. However, this is as far as the resemblance goes. The Bird, it now appears, has no true blastopore related to the embryo itself, and the so-called primitive streak, if homologous with a blastopore, is formed in a different manner from any of the streaks so far described.

THE DIFFERENTIATION OF MESODERM, NOTOCHORD,
AND DEFINITIVE ENDODERM

By means of the above processes of epiboly and involution, there is presently developed a telolecithal *gastrula*, whose lower or endodermal layer forms a roof for the former segmentation cavity (now the *archenteron*) in much the same way as in the Teleosts. In the present case, also, this layer soon gives rise to the *mesoderm* and *notochord*. The lat-

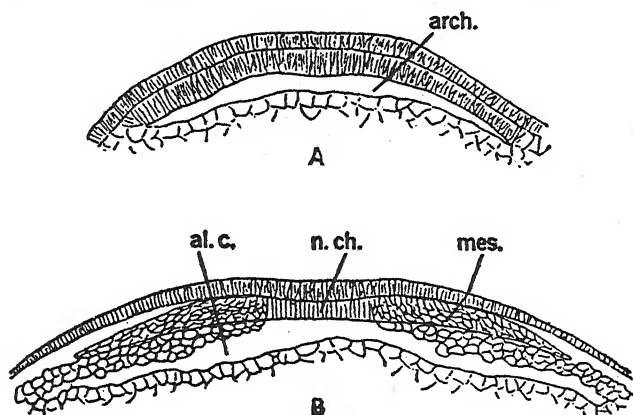


Fig. 150. — Transverse sections of *Hypogeophis* showing the differentiation of the roof of the archenteron into notochord (*n.ch.*) and mesoderm and the formation of the gut (*al.c.*) by upgrowth of yolk-cells from the sides. From Jenkinson (*Vertebrate Embryology*). After Brauer.

ter originates quite as in the Fish, but the formation of the mesoderm differs in the way previously noted as characteristic of other Urodeles. Thus in the Teleost it will be recalled that, though the development of the notochord involved all of the hypoblast in the median line of the embryo, the mesoderm on either side was merely split off, leaving a layer of endoderm beneath it. In the Gymnophiona, on the other hand, the entire central portion of the archenteric roof which did not go to form the notochord becomes mesoderm (Fig. 150). There is no delamination, and the result is that within the central area of the blastoderm, the enteric cavity for the time being is roofed only by mesoderm and notochord. In other words, in this case the central portion of the mesoderm, as well as the notochord, consumes in its formation all of the hypoblast beneath it. Presently, however, the endoderm in this central region is supplied by the ingrowth of lower layer cells from about the margin (Fig. 150). The uppermost layer as usual is now termed *ectoderm* and,

as in the forms previously studied, all three layers are continuous with one another about the lips of the blastopore.

As will presently appear the methods of mesoderm and notochord formation in the Teleosts and Gymnophiona are not particularly significant as regards an understanding of these processes in the Bird. Yet, because as usual, their occurrence somewhat overlaps gastrulation as strictly defined, an account of their character has been included for the sake of completeness.¹

REFERENCES TO LITERATURE

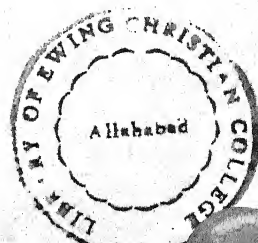
CHAPTER VII

- Brauer, A., "Beiträge zur Entwicklungsgeschichte der Gymnophionen," *Zool. Jahrb.*, X, 1897.
- Brummett, A. R., "The relationships of the germ ring to the formation of the tail bud in *Fundulus* as demonstrated by the carbon marking technique," *Jour. Exp. Zool.*, 1954.
- Hertwig, O. (Editor), *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere*, I, 1, 1, "Die Lehre von den Keimblättern," Jena, 1903 (1906).
- Hertwig, O. and R., "Studies on the Germ Layers," *Jena Zeitschr.*, XIII-XVI (VI-IX), 1879-1883.
- His, W., "Untersuchungen über die Entwicklung von Knochenfischen, besonders über diejenige des Salmens," *Zeit. Anat. Entw.*, I, 1876. — "Untersuchungen über die Bildung des Knochenfischembryo," *Arch. Anat. u. Entw.*, 1878.
- Jenkinson, J. W., *Vertebrate Embryology*, Oxford and London, 1913.
- Kopsch, F., *Untersuchungen über Gastrulation und Embryobildung bei den Chordaten*, "I. Die Morphologische Bedeutung des Keimhautrandes und die Embryobildung bei der Forelle," Leipzig, 1904.
- Korschelt and Heider, *Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere*, I, "Experimentelle Entwicklungsgeschichte," Jena, 1902. — *Lehrbuch, etc.*, III, "Furchung und Keimblätterbildung," Jena, 1909-1910.
- McEwen, R. S., "The Early Development of *Hemichromis bimaculata* with Special Reference to Factors Determining the Embryonic Axis," *Jour. Morph. and Physiol.*, XLIX, 1930.
- Oppenheimer, J. M., "Processes of localization in developing *Fundulus*," *Jour. Exp. Zool.*, LXXIII, 1936. — "Potencies for differentiation in the teleostean germ ring," *Jour. Exp. Zool.*, LXXIX, 1938.
- Sumner, F. B., "Kupffer's Vesicle and its Relation to Gastrulation and Convergence," *Mem. N. Y. Acad. Sci.*, II, 1900. — "A Study of Early Fish Development: Experimental and Morphological," *Arch. Entw.-mech.*, XVII, 1903.
- Wilson, H. V., *The Embryology of the Sea Bass (*Serranus atrarius*)*, (Bull. U. S. Fish Commission, IX), 1889.

¹ Brummett '54 has made a study of gastrulation in the fish, *Fundulus*, marking the blastodermal margin (germ ring) with carbon particles instead of stain, and concludes that, somewhat contrary to Oppenheimer and others, there is very little confluence or convergence in this form. Only the regions of the ring at, and quite near (less than 90 degrees from) the incipient dorsal lip, are involved, and they form only the extreme posterior of the embryo and tail bud.

PART IV

THE DEVELOPMENT OF THE CHICK



T

HE CHICK: THE ADULT REPRODUCTIVE ORGANS, AND THE DEVELOPMENT OF THE EGG PREVIOUS TO GASTRULATION

THE Chick has long been an object of embryological interest, and the study of its development has been connected with such classical names as Malpighi (1672), Wolff (1759), and Von Baer (1828). In the more modern era of science, moreover, workers in this field have continued to study it, until at the present time probably more details regarding its development are known than in the case of any other animal. As will appear, however, certain points concerning the very early stages are even yet in doubt, and are still under investigation.

Some of the reasons for the importance of this form and the study which has been given it may be briefly indicated. In the first place the material is usually easy to obtain and observe throughout most of the developmental stages. Furthermore, unlike the Frog or Fish, the Chick embryo, in common with those of other Birds as well as with those of Reptiles, possesses certain very significant extra-embryonic membranes and appendages. The significance of these structures lies not only in their character and functions in the groups just cited, but also in the fact that the same appendages and membranes occur also in the Mammals, though in a somewhat modified condition. Lastly, aside from the features already indicated, the general development of the Chick is more nearly mammalian than that of any of the forms previously considered.

In the following account we shall begin with a brief description of the reproductive organs of the adult Bird.

REPRODUCTIVE ORGANS OF THE ADULT, OÖGENESIS AND OVULATION

THE MALE

The male Bird, or Cock, possesses a pair of testes, each of which is an ellipsoidal body about two inches long and one inch in diameter.

It is made up of *seminiferous tubules* and supporting tissue, and, as in the case of the Frog, is rather closely attached to the dorsal wall of the coelom by a fold of coelomic epithelium, the *mesorchium*. By way of the *vasa efferentia*, each testis discharges its products into its respective

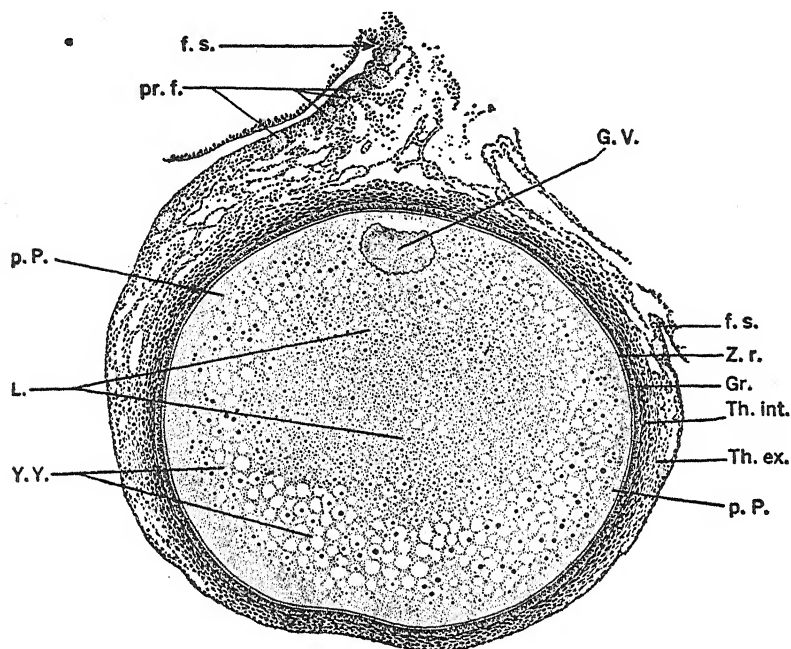


Fig. 151. — Section of an ovarian ovum of the Pigeon, drawn from a preparation of Mr. J. T. Patterson. From Lillie (*Development of the Chick*). The actual dimensions of the ovum are 1.44 x 1.25 mm.

f. s. Stalk of follicle. *G. V.* Germinal vesicle. *Gr.* Granulosa. *L.* Latebra. *p. P.* Peripheral protoplasm. *pr. f.* Primordial follicles. *Th. ex.* Theca externa. *Th. int.* Theca interna. *Y. Y.* Yellow yolk. *Z. r.* Zona radiata.

vas deferens. The latter duct then leads to the cloaca, where its entrance is marked by a papilla. There is some evidence that the sperm attain their motility and functional capacity by the action of a testis hormone during their passage through the *vasa efferentia* (Munro, '38).

THE FEMALE

The Ovary. — In the embryo Chick two ovaries are present, but only the left develops. In the adult Fowl this is suspended from the

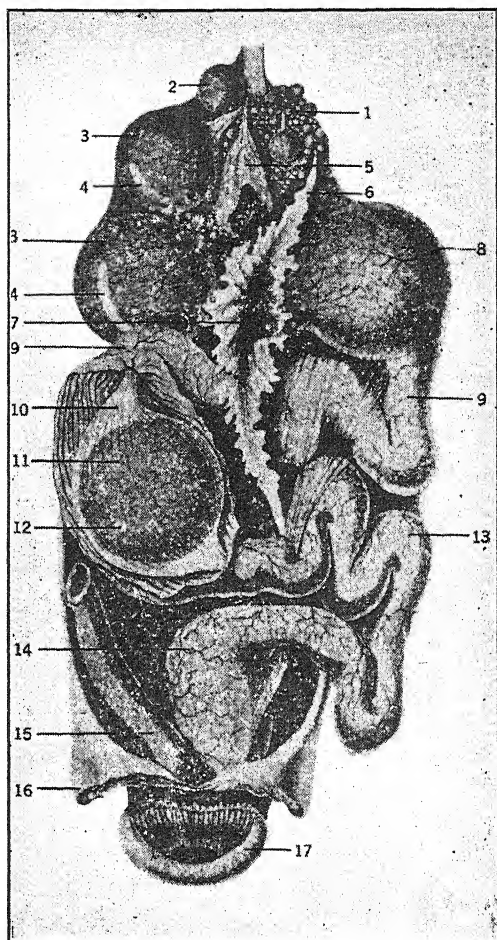


Fig. 152. — Reproductive organs of the Hen. (After Duval, based on a figure by Coste). From Lillie (*Development of the Chick*). The figure is diagrammatic in one respect, namely, that two ova are shown in the oviduct at different levels; normally but one ovum is found in the oviduct at a time.

1. Ovary; region of young follicles. 2 and 3. Successively larger follicles. 4. Stigmata (cicatrices), or non-vascular areas, along which the rupture of the follicles takes place. 5. Empty follicle. 6. Cephalic lip of ostium. 7. Funnel of oviduct (ostium tubae abdominale). 8. Ovum in the upper part of the oviduct. 9. The magnum, where most, if not all, the albumen is actually secreted. 10. Albumen surrounding an ovum. 11. Ovum in portion of duct laid open to show it. 12. Germinal disc. 13. The isthmus where the shell membrane is secreted, and possibly some thin albumen. 14. The uterus where shell is secreted, and both layers of thin albumen separated from remainder, producing thick albumen and chalazae (see text). 15. Rectum. 16. Reflected wall of abdomen. 17. Anus, or external opening of cloaca.

body wall by the *mesovarium* in about the same position as the left testis in the male. It consists of the usual vascular connective tissue elements, or *stroma*, within which are imbedded ova in various stages of growth. Each ovum is surrounded by a layer of *follicle* or *granulosa cells*, and these in turn are encased in a sheath of the stroma called the *theca*. It is sometimes customary to refer to such stages together with their coverings as simply *follicles* (Fig. 151). Normally only one ovum matures at a time, though there may be several not many hours apart.

The Genital Tract.—As in the case of the ovary, only the left genital tract develops. This fact is apparently correlated with the production by Birds of fragile shelled eggs, such that the coming together of two at the cloaca would be disastrous. In this connection it is of some interest to note that although in certain species of Hawks there are two fully developed ovaries, there is still only one genital tract (Stanley and Witschi, '40). As regards this tract, we find that it opens anteriorly adjacent to the ovary and posteriorly into the cloaca just dorsal to the anus. Also it is suspended as usual from the dorsal body wall by a mesentery-like fold of peritoneum, and in the Birds it may be divided into three main parts as follows:

I. The Oviduct Proper. This is the anterior part and is itself divisible into three sections:

(a) *The Infundibulum or Ostium.* This is a thin-walled muscular funnel, the inner surface of which is lined by ciliated epithelium. It is in the immediate neighborhood of the ovary, but does not directly connect with it.

(b) *The Magnum.* This is sometimes called the "glandular portion," but since other parts are also glandular this is not a very good designation. The part in question is a long much convoluted tube following immediately after the ostium. It leads into:

(c) *The Isthmus.* This is a shorter tube also glandular whose posterior end marks the termination of the oviduct proper.

II. The Uterus. This is a relatively short, dilated portion whose walls are also glandular. It immediately follows the isthmus and leads into the third and last main division:

III. The Vagina. This region is likewise short, but thin-walled, and opens into the cloaca (Fig. 152).

The Oögonia.—The origin of the primordial germ cells and their multiplication as oögonia occur during the embryonic life of the Chick. This early history will therefore be dealt with later in connection with the development of the gonads. At the time of hatching, however, the

oögonia are said to have ceased to divide, and each is becoming surrounded by follicle cells preparatory to growth (Fig. 153). They may now, therefore, be called oöcytes, or young ova, whose history from this point onward will be taken up in more detail.

The Growth Period.

The Vitelline Membrane or Zona Radiata. — There now appears surrounding each ovum or oöcyte a membrane which is called the *vitelline membrane*. Whether it is a true vitelline membrane arising entirely from the surface of the egg itself, or whether it is secreted by the follicle cells and is therefore chorionic in character, is somewhat uncertain. As this membrane thickens slightly, it becomes pierced by minute canals; for this reason it is also referred to sometimes as the *zona radiata*. Throughout these canals by way of the follicle cells the egg receives nourishment from the surrounding theca.

The Germinal Disc. — At first the nucleus occupies the center of the oöcyte, and the yolk granules are deposited in the cytoplasm around it. This presently results in the existence of yolk-free cytoplasm only around the periphery of the egg. This cytoplasm, however, is thicker upon the side where the theca of the ovum is attached to the ovary; this thickening is called the *germinal disc* (*blastodisc*). Meanwhile the ovum has been growing, and by the time it has become .6 mm. in diameter, the nucleus has migrated into this disc (Fig. 151).

The Deposition of Yolk. — The growth of the ovum is largely due to the deposits of yolk, which it appears occur in the following manner: The nucleus, as noted, occupies at first a central position around which the yolk begins to be formed. This yolk is of a lightish color termed *white yolk*, and the central mass of it which is thus deposited is known as the *latebra*. Following this the peripheral layer of the protoplasm starts to deposit around the latebra a darker colored substance, the *yellow yolk*. As the egg is thus enlarged, the nucleus, as indicated, leaves its central location and takes a peripheral position, which it maintains during subsequent growth. The result is that the yellow layer is everywhere interrupted along the path which the nucleus has taken. Along this path there is thus left a continuous deposit of white yolk extending from the latebra almost to the surface. It is known as the *neck* of the latebra, and just beneath the blastodisc it spreads slightly to form a plate, the *nucleus of Pander* (Fig. 154, B).

It should be noted that in some instances the deposit of yellow yolk is interrupted by intermittent, usually thinner, layers of more white

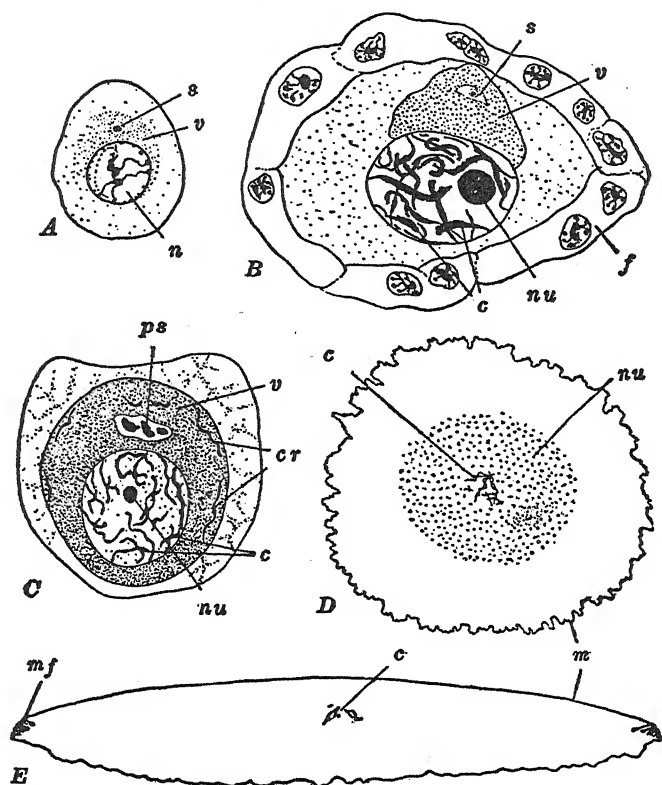


Fig. 153. — Growth stages in the oögenesis of the Hen's egg. From Kellicott (*Chordate Development*). After Sonnenbrodt. A. Oöcyte measuring 0.012×0.016 mm., the nucleus of which is 0.006 mm. in diameter. B. Oöcyte measuring 0.018×0.023 mm., the nucleus of which is 0.0105×0.014 mm., enclosed in follicle. C. Oöcyte measuring 0.040×0.045 mm., the nucleus of which is 0.020×0.022 mm. D. The nucleus only, of an oöcyte measuring 5.84×6.16 mm., the nucleus itself measuring 0.214×0.238 mm. Total view showing the small chromosomes in the midst of a collection of chromatin nucleoli. E. Vertical section of the nucleus only, of an oöcyte, the follicle of which measured 37 mm. in diameter. The nucleus itself is 0.455 mm. in diameter and 0.072 mm. in greatest thickness.

c. Chromosomes. cr. Extra nuclear chromosome-like bodies. f. Follicle. m. Nuclear membrane. mf. Folds in nuclear membrane. n. Nucleus. nu. Chromatin nucleolus. ps. Pseudo-chromosomes. s. Centrosome. v. Yolk nucleus or vitellogenous body.

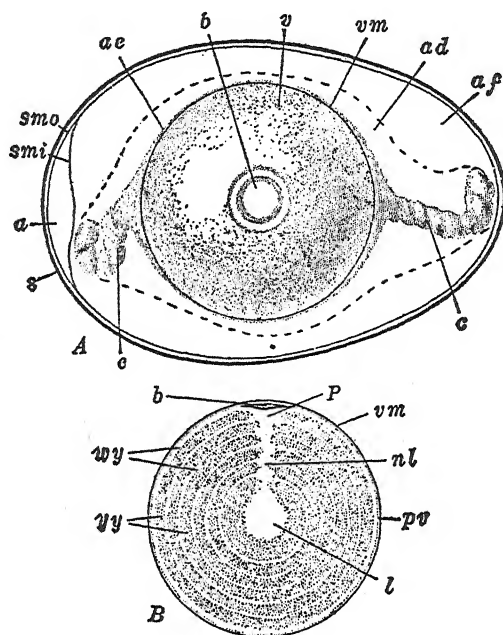


Fig. 154. — Semidiagrammatic illustration of the Hen's egg at the time of laying. From Kellicott (*Chordate Development*). A. Entire "egg." Modified from Marshall. B. Vertical section through the vitellus or ovum proper, showing the concentric layers of white and yellow yolk. Actually there are seldom, if ever, as many layers as this under normal conditions.

a. Air chamber. ac. Chalaziferous layer of albumen. ad. Dense layer of albumen. af. Fluid layer of albumen. b. Blastoderm. c. Chalaza. l. Latebra. nl. Neck of latebra. p. Nucleus of Pander. pv. Perivitelline space. s. Shell. smi. Inner layer of shell membrane. smo. Outer layer of shell membrane. v. Vitellus or "yolk." vm. Vitelline membrane. wy. Layers of white yolk. yy. Layers of yellow yolk.

yolk. This alternation was once thought to be universal, and to result from the fact that yellow yolk was deposited during daylight and white yolk at night (Fig. 154, B). As indicated, however, many eggs can be found in which no such alternation of layers exists, all the yolk aside from the latebra and its neck being yellow. Experiment has now shown that the differences in color of the layers, when they occur, are due entirely to alternating differences in the character of the food. The deeper yellow is produced by xanthophyl, and appears in the yolk when grass

or yellow corn occurs in the diet. If this is fed periodically, it results in an alternation of darker and lighter layers. Thus by proper feeding thick or thin, few or numerous, layers can be produced at will. The white yolk of the latebra and its neck, however, always occurs, and is evidently of a different character. It apparently results from some influence of the nucleus, but its cause is unknown (Conrad and Warren, '39).

OVULATION, MEIOSIS, AND FERTILIZATION

During these processes the nucleus has greatly enlarged and as usual in its enlarged form it is known as the *germinal vesicle*. The first maturation division is initiated about $4\frac{1}{2}$ hours previous to ovulation, and is completed in about $2\frac{1}{2}$ hours, after which the spindle for the second division is formed (Olsen, '42, '50). At this point the large ovum still in the ovary is grasped by the funnel shaped infundibulum or ostium. The theca and follicle then rupture along a non-vascular line, the *cicatrix*, and the egg is received into the oviduct.

Finally it may be noted that occasionally two eggs may mature and be released together, in which case they are enclosed in a single shell and form a "double yolk egg." While this is apparently the most usual cause of this condition it is not the only one. Such eggs may also result either from the premature or the late ovulation of one of the "yolks" (eggs), or from the picking up by the infundibulum of an extra egg which has previously fallen into the body cavity.

FERTILIZATION AND MEIOSIS

When the egg is taken into the ostium, it is at once surrounded by sperm which have been received from the male at a period from 24 hours to two weeks previous to the ovulation of the ovum in question. Several sperm enter the egg presumably, as in the Pigeon, in the neighborhood of the blastodisc, following which the second polar body is given off and the egg pronucleus fuses with that of one of the sperm. Many of the remaining sperm nuclei then degenerate, while others (*supernumerary nuclei* or *merocytes*) persist for a time and produce certain phenomena to be described later in connection with segmentation.

THE HISTORY OF THE OVUM FROM FERTILIZATION THROUGH GASTRULATION

The stages now to be described have not all been completely worked out for the Chick. It is presumed, however, that they are somewhat

similar to the corresponding stages in the Pigeon which have been fully described by Patterson and Blount. Data concerning doubtful stages in the Hen's egg have therefore been partially supplied from the facts regarding the Pigeon. The points where this has been done will be noted in passing.

THE APPLICATION OF ALBUMEN, SHELL MEMBRANES AND SHELL

Strictly speaking, the formation of the ovum proper is completed at the time of ovulation, and it thus appears that what is ordinarily spoken of as the "yolk" of the Hen's egg is really the entire egg. Nevertheless, in the case of the Bird, it is common usage to include under the term egg not only the ovum proper (i.e., the "yolk") but also all its tertiary membranes, and this usage will be adhered to in the following account:

As the yolk passes down the oviduct it takes a position such that a line passing through the blastodisc and the center of the vegetal pole is at right angles to the longitudinal axis of the duct at any particular point. It then revolves slowly about the latter axis, and while so doing receives its respective coverings from certain portions of the duct. In the completed product these coverings of the egg or "yolk" are as follows:

Closely applied to the yolk comes a dense layer of albuminous substance filled with fine mucin-like threads. This layer forms a thin but firm covering, the *chalaziferous membrane*. At each side of the yolk opposite each end of the shell this membrane is twisted into cords, the *chalazae*. Immediately outside of this chalaziferous membrane there is said to occur a very narrow layer of thin watery albumen (Conrad and Scott, '38). There then comes a clear but relatively dense and wide layer of albumen called simply *dense albumen*. Its density is apparently also due to the presence of mucin. This layer in turn is surrounded by a fairly wide layer of thin watery albumen called *thin albumen* which is bounded externally by the so-called *shell membrane*. The latter is a very real and definite membrane in immediate contact with the outermost coverings of all, the calcareous *shell*. The chalazae and the wide layers of dense and of thin albumen are easily demonstrated by carefully breaking an uncooked egg into a finger bowl. The innermost narrow layer of thin albumen next to the chalaziferous membrane, however, is not usually seen except by the use of more refined methods. The shell membrane is readily detectable sticking to the inside of the shell. In a hard-boiled egg the albumen can be more or less unwound in spiral

sheets, apparently a result of the revolving of the egg in the duct during its application. (Fig. 154, A).

The question now arises as to what parts of the genital tract listed above are responsible for the different layers and membranes just indicated. This has been investigated by various workers, Asmundsen and Burmester ('36), Burmester ('40), Cole ('38), Conrad and Phillips ('38), Scott and Wai-Lan Huang ('41) and others. These men have attacked the problem by removing parts of the duct to see what layers were reduced or lacking, by studying the histology of parts of the tract and in other ways. While the results of their investigations are not in entire agreement on some details the following conclusions taken largely from the discussion of Conrad and Scott ('38) are probably very near to the truth.

Products of the Magnum. — The egg having taken about 18 minutes to pass the infundibulum enters the magnum which it goes through in a little short of three hours.¹ This latter region secretes all of the thick or *dense albumen* which owes its character to numerous mucin threads. Some (Asmundsen) claim that a little thin albumen (that of the narrow layer?) is also secreted by the anterior part of the magnum, but this seems to be one of the points on which there is disagreement (see below).

Products of the Isthmus. — The egg passes through this part of the duct in about 74 minutes, and receives here the *shell membrane*. There may also be a little thin albumen secreted by this part of the duct, though Conrad and Scott claim that almost all, if not all, of this is produced, i.e., differentiated from other materials, while the egg is in the uterus. As will presently appear, however, not all the constituents of this albumen are actually secreted in the latter organ.

Products of the Uterus. — The egg remains longest of all in this region, about $20\frac{1}{2}$ hours, and as just suggested it is while the egg is here that practically all of the *thin albumen* is differentiated as such. As noted, however, all of the material for this layer does not actually originate in this part of the tract. Instead that portion of it which does arise here consists largely of thin non-albuminous fluid and soluble salts. This solution of salts then passes by osmosis through the already existent shell membrane which is thereby distended. When the fluid in question thus comes next to the dense albumen some of the protein in the latter, other than the mucin, soon diffuses into the fluid. In this

¹ Average time spent in various parts of the duct was kindly furnished by Dr. D. C. Warren.

way the latter becomes albuminous, though still thin because it lacks mucin threads.

While the egg is in the uterus there are also produced the *chalazae*, *chalaziferous membrane* and the *narrow layer of thin albumen*. In this case, however, none of the materials concerned are secreted here. The substances for these structures are already present in the dense albumen produced in the magnum. What happens is this: The mucin fibers in the part of the thick albumen immediately adjacent to the yolk are withdrawn from this albumen, and are concentrated against the yolk to form the chalaziferous membrane. This concentration leaves the albumen next to the membrane without any fibers, and hence it becomes thin, thus forming the very narrow thin layer noted as occurring in this region. The chalazae are simply extensions of the concentration at the two sides of the yolk. They are twisted apparently because the egg was rotating at the time the albumen from which they are derived was laid down, and possibly because rotation is still going on. The cause of the separation of the mucin from the albumen is believed to be mechanical, but the process is not entirely clear.

Finally the *shell* is entirely secreted by the uterus, and is known to be substantially advanced, though not completed, after 8-10 hours within that part of the genital tract. The source of the cuticle of the shell is uncertain, but it may be denatured protein.

The Vagina. — The egg probably remains only a few seconds in the vagina before it is laid, and there is nothing added to it here.

THE PERIODICITY OF LAYING

The periodicity in the laying of eggs has been a subject of considerable investigation. Most chickens have an annual laying period of eight or nine months, the commonest interval of rest being during the late summer months. During the active period the Bird lays more or less continuously at the rate of about an egg a day, if the eggs are constantly removed. Otherwise when a sufficient number have been accumulated the impulse to "set" may assert itself, and the laying ceases while a brood is hatched and raised. From this it might be inferred that the impulse to set is dependent merely upon the accumulation of a certain number of eggs, but the word "may" in the previous sentence is used advisedly. Not every hen will set when enough eggs are accumulated. On the other hand, the setting impulse, i.e., "broodiness," sometimes asserts itself whether there are eggs or not. This is most likely to happen in the spring and early summer, i.e., during the time of year which

is the breeding season of many birds in temperate latitudes. Thus the impulse to set is evidently due to more than the single factor of egg accumulation. It is probably, like so many aspects of reproduction, partly controlled by some of the endocrine glands, particularly the pituitary, and this in turn may well be influenced by the length of day, the temperature, or both. This irregularity in the advent of broodiness in domestic hens is very likely the result of long selection with a view to increasing the laying period. Even if the eggs are removed, however, and the hen does not become broody, she does not lay one every day for an indefinite period. Instead she lays a series of eggs on successive days, and then skips a day, such an uninterrupted series being known as a *clutch*. The eggs of a clutch, moreover, are not laid at the same time each day. Rather the first one will be laid fairly early in the morning of the first day, and each succeeding one about two hours later than its predecessor on each of the following days. This continues until the last egg of the clutch is laid around the middle of the afternoon, seldom later. This means that after a maximum of five or six eggs has been laid, a day will ensue in which none is laid, and the hen will then begin again in the morning of the day following.

It was formerly believed that this interruption in laying was due to a delay in the act of laying itself. The theory was that if an egg was not ready to be laid until late in the afternoon, the Bird would not lay it then, but would retain it over night. Thus a day would pass with no egg laid and the one laid the following morning would be a so-called "held egg." This idea was made reasonable by the fact that there is some difference in the degree of development of eggs, and this assumed opportunity for prelaying incubation was supposed to account for it. Further study, however, has rendered this theory untenable. In the first place careful tracing of the history of eggs in the genital tract proves, according to Scott and Warren ('36) that there are no held eggs. Instead it has been found that all eggs spend approximately 25 hours in the genital tract with some minor variations. It is thought that these minor variations are sufficient to account for such differences in embryonic development as are known to occur. Correlated with this near equality of time spent in the tract is the fact that each egg in a clutch is ovulated within a few minutes of the laying of the previous one of that clutch. These considerations would suggest that the explanation for the omitted day must lie either in delay of ovulation of completely formed eggs, or in a delay in the later growth stages of certain eggs in the ovary.

An effort to find which of the latter suppositions is true, and to determine the cause for whatever delay may occur, has been made by subjecting the hens to variations in illumination. It has thus been found that artificially reversing the time of illumination within the 24-hour period will cause a corresponding reversal in the time of laying, but this effect is delayed for about sixty hours. Also constant illumination will cause the hens to distribute their laying more or less regularly throughout the 24-hour period, and will make them lay more eggs to a clutch. Clutches, however, do still occur, i.e., the laying is not continuous. This and other data led Warren and Scott ('36) to conclude that illumination is responsible for normal periodicity in laying. Furthermore since there are no held eggs the influence of the light could not be upon the laying itself. It must be upon earlier stages in the entire process.

Finally because of the time lag before changed conditions produced results these authors decided that the influence was also not upon ovulation, but, as intimated above, upon late stages in the growth of the oöcyte. Be this as it may, still later investigations by Fraps, Neher and Rothechild ('47) have shown that light is not the only environmental factor involved. By giving or withholding food during continuous illumination it was clearly shown that this item and the accompanying activity of obtaining it very definitely stimulate some step in the reproductive process, apparently ovulation. Also as was so thoroughly demonstrated in the Frog, pituitary secretion seems to be the immediate internal agent through which the external factors act.

SEGMENTATION

While the egg has been passing down the oviduct and receiving its outer coverings, segmentation has been practically completed. As in the Teleost and Gymnophiona eggs, this process involves only the germinal disc (blastodisc), which at the time of the first cleavage is about 3 mm. in diameter and 0.5 mm. thick. It takes place in the following manner and in the parts of the duct indicated:

The First Cleavage. — The first cleavage furrow forms in about the middle of the blastodisc, and extends only part way across it and part way through it. It is completed during the passage of the magnum (Fig. 155, A).

The Second and Third Cleavages and the Accessory Cleavage. — As the egg enters the isthmus the second cleavage furrow begins to form in the two existing cells; it is approximately perpendicular to the middle of the first furrow, and is of about the same depth. There

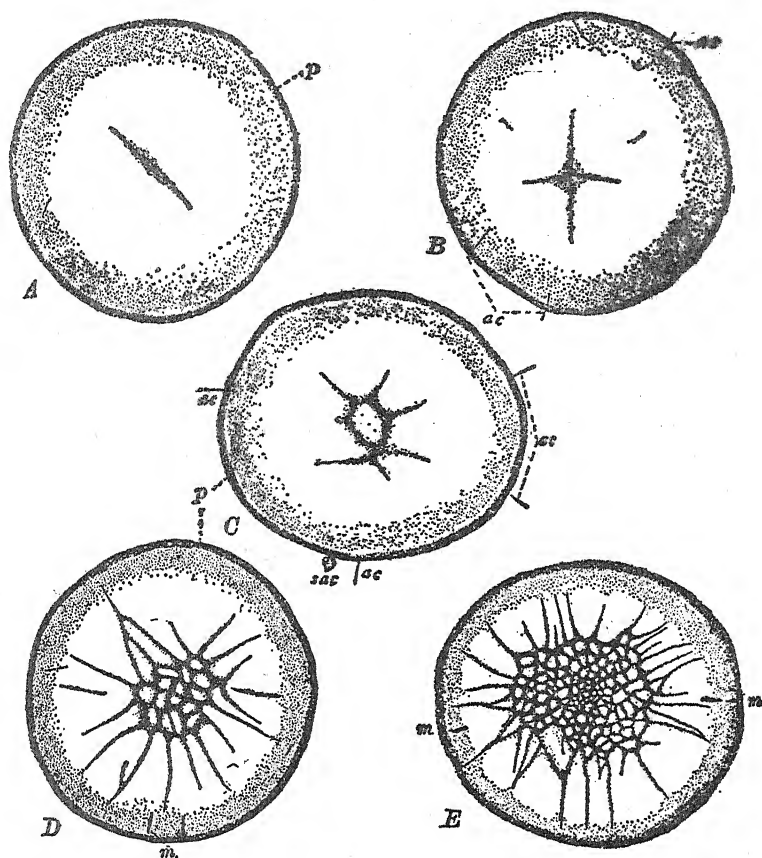


Fig. 155. — Cleavage in the Hen's egg. Surface views of the blastoderm and the inner part of the marginal periblast only. From Patterson. The anterior margin of the blastodisc is toward the top of the page. *A*. Two cell stage about three hours after fertilization. *B*. Four cells, about three and one-fourth hours after fertilization. *C*. Eight cells, about four hours after fertilization. *D*. Thirty-four cells, about four and three-fourths hours after fertilization. *E*. One hundred and fifty-four cells upon the surface; the blastoderm averages about three cells in thickness at this stage (about seven hours after fertilization).

ac. Accessory cleavage furrows. *m*. Radial furrows. *p*. Inner part of marginal periblast. *sac*. Small cell formed by the accessory cleavage furrows.

thus arise four cells, in each of which the furrow of the third cleavage soon appears. These third cleavage furrows may be parallel with the first, but their direction is quite frequently irregular. In this manner

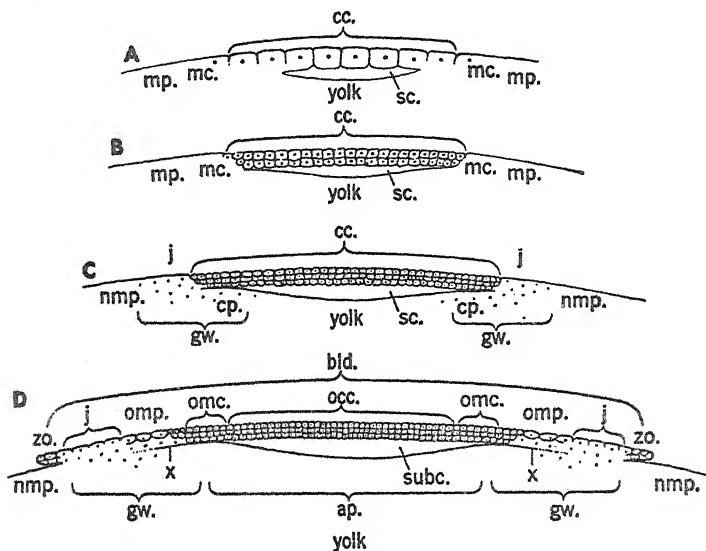


Fig. 156. — Diagrams of vertical sections through the blastoderm of a Hen's egg during cleavage stages. *A*. A section through an approximate 32 cell stage. *B*. A section through a slightly later stage where marginal cells are being added to the original central cells. *C*. A section through a still later stage in which the marginal cells have all been used up, and the extra (periblast) nuclei from some of them are invading the periblast to form the germ wall. *D*. A stage just as gastrulation is about to begin. The zones of junction and overgrowth are well marked, and the germ wall is beginning to add cells to the original marginal cells.

ap. Approximate extent of the area pellucida, not yet marked, however, by the thinning of the blastodermal roof. *bld.* Blastoderm. *cc.* Central cells. *cp.* Central periblast. *gw.* Germ wall. *j.* Zone of junction. *nmp.* New marginal periblast. *mc.* Marginal cells. *mp.* Marginal periblast. *occ.* Original central cell region. *omc.* Original marginal cell region. *omp.* Original marginal periblast region. *sc.* Segmentation cavity. *subc.* Subgerminal cavity. *x.* Line of separation between the inner portion of the germ wall and the underlying yolk. *zo.* Zone of overgrowth.

eight cells are formed, none of which are at first separated from the deeper protoplasm of the disc or from that at the margin.

Before continuing the account of the regular cleavages it is now necessary to pause a moment to note certain so-called *accessory cleavages*. These cleavages, which are extremely slight and transitory in the Hen's egg, seem to result from a few divisions of some of the supernumerary sperm nuclei indicated above. They appear at about the four-cell stage as faint radial furrows around the edge of the blastodisc, but by the

time ten cells have formed they have completely vanished. Scattered and degenerating sperm nuclei are sometimes observable as late as the thirty-two-cell stage; these also, however, are presently lost sight of, and apparently exercise no influence upon the ovum (Fig. 155).

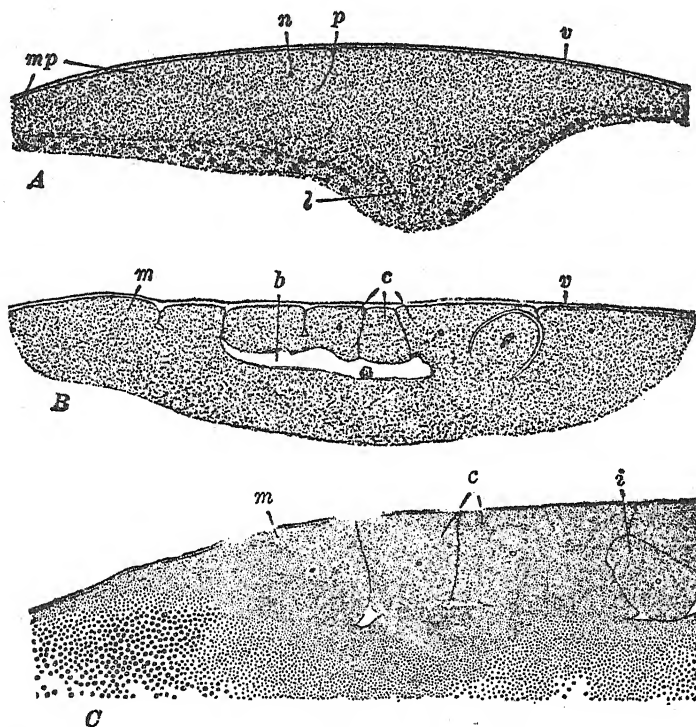


Fig. 157. — Vertical sections through the Chick blastoderm during the process of cleavage. From Kellicott (*Chordate Development*). After Patterson. A. Section through the two cell stage. B. Median section through the thirty-two cell stage. C. Part of a longitudinal section through the sixty-four cell stage.

b. Blastocoel or segmentation cavity. c. Central cells. i. Inner cell cut off by horizontal cleavage. l. Neck of latebra. m. Marginal cells. mp. Marginal periblast. n. Nucleus. p. First cleavage. v. Vitelline membrane.

The Central and Marginal Cells. — Subsequent to the eight-cell condition, following the third cleavage, further furrows soon appear, which result in the production of approximately sixteen cells. Some of these furrows, moreover, are such as definitely to bound the outer edges of those cells, whose protoplasm has heretofore been continuous with that which lay further out. Hence, there is thus created a central seg-

mented area completely delineated from the unsegmented protoplasm about it; the cells of this area are termed the *central cells*.

Cleavage then continues about the rim of this central area, producing new cells here which because of their position are called *marginal cells*. These cells are for the time being unseparated both from the yolk filled cytoplasm beneath, and from that lying still further toward the periphery. This condition is characteristic of what is later known as the zone of junction (see below). As the process of cleavage goes on these marginal cells are constantly being cut off and added to the central cells; meanwhile beyond them more marginal cells arise. In this manner the central segmented area is continually increasing in diameter (Fig. 156, A; Fig. 157).

The Segmentation Cavity. — Furthermore, at the same time that the central cells are being defined as such by the furrows at their margins, horizontal cleavages are also taking place. These cleavages intersect the furrows which are visible from the surface, and thus cut off a single superficial layer of the central cells from the protoplasm beneath them. Fluid then begins to collect between this layer of cells and the protoplasm, thus establishing a shallow space, the rudiment of the *segmentation cavity*.

As the egg leaves the isthmus, there have been formed in this manner approximately thirty-two cells; ² it next enters the uterus, in which cleavage is completed and gastrulation begun.

The Periblast and Its Segmentation. — Further division both horizontal and otherwise now takes place, so that the layer of central cells, at first only one cell thick, soon acquires a thickness of several cells; the area covered by the central and marginal cells has likewise been increased. All the cleavage thus far indicated, however, has taken place within the central region of the blastodisc (Fig. 156, B). About the margin of this area, there remains a ring of the disc slightly darker in color than the central portion, and about .5 mm. wide. It is still entirely unsegmented and is known as the *periblast*.

The Germ Wall and Subgerminal Cavity. ³ — Presently the formation of marginal cells about the edge of the central region reaches to the inner margin of the ring, defined as periblast. At this point, although the nuclei of the marginal cells continue to divide, the cytoplasmic

² There are, according to Kölliker, about forty-four cells in the blastoderm of the Chick at this stage.

³ The ensuing description of the organization of the periblast and other later phases of segmentation are from the accounts of Blount and Patterson, of homologous processes in the Pigeon.

cleavages do not keep pace with them. The extra nuclei (*periblast nuclei*) thus produced then wander out into the region of the periblast and convert it into a syncytium. Some of these nuclei even move centrally for a short distance into the unsegmented protoplasm beneath the rim of the segmentation cavity. The latter region of protoplasm thus occupied by the extra nuclei is usually known as the *central* or *subgerminal* periblast (see below), to distinguish it from the strictly marginal periblast, the two regions, however, being perfectly continuous. Following the above-mentioned penetration by the periblast nuclei, what was periblast both central and marginal, is known as *germ wall*, the peripheral non-nucleated cytoplasm in turn becoming periblast (Fig. 156, D). Meanwhile, the last of the original marginal cells have been cut off from the outlying periblast (now germ wall), and have become continuous with, and similar in character to, the cells originally defined as central. Within the syncytial germ wall, cytoplasmic cleavage next begins to take place, and the cells which are thus produced are added to the former marginal cells. Thus, partly by the multiplication of the cells already in existence, and partly by the peripheral addition of new cells arising within the wall, the central area of completely defined cells spreads outward over the surface of the yolk. Upon this basis it might be imagined that the germ wall would soon be used up, and as regards the portion of it defined as central periblast this appears to be true. The marginal part of the wall, however, is never exhausted during this process of overgrowth. This is due to the fact that as fast as its inner margin becomes nucleated and then converted into cells, a new germ wall is created by the peripheral movement of more periblast nuclei into the new periblast region which lies continually further out. Meanwhile, as the cellular area is thus extended, the original segmentation cavity likewise enlarges beneath it. This augmented central space is then often referred to as the *subgerminal cavity*,⁴ whose outward extension as such ceases about the time gastrulation is completed.

The Zone of Junction and the Zone of Overgrowth.—Beyond the extent of the subgerminal cavity, however, the cellular area continues to spread over the yolk. Although the actual cavity as such ceases to expand subsequent to gastrulation, this outgrowth of the cellular region is accompanied by an ever-widening zone, in which the newly formed cells are nevertheless distinctly separated from the underlying yolk. The separation is then continuous at its inner margin with the subger-

⁴ The above distinction between segmentation cavity and subgerminal cavity is frequently not adhered to, the two terms being considered synonymous.

minal cavity. It should further be noted that at its outer edge this zone of separation extends somewhat beyond the region where the germ wall has been entirely organized, within its deeper portions, into cells. In other words at the inner margin of the germ wall, the latter is already slightly separated from the yolk beneath it (Fig. 156, *D*, *x*). In its more peripheral part, on the other hand, the germ wall, as already indicated, is quite continuous with the underlying yolk. Likewise, the cells which, even in this outer zone, now cover the upper surface of the wall as fast as it forms, are unseparated by cytoplasmic cleavage from the unsegmented portion of the wall beneath them. Because of this lack of separation between these superficial cells and the wall beneath them, and also between the wall and the underlying yolk, this outer portion of the germ wall is known as the *zone of junction* (Fig. 156, *D*). Lastly, beyond the extreme limit of the zone of junction there exists a narrow superficial rim of cells which extends out over the unsegmented yolk (periblast), from which it is quite separate. This is called the *zone of overgrowth*, and, although arising from the outer edge of the zone of junction, it seems to be maintained by the multiplication of its own cells (Fig. 156, *D*).

The Blastoderm. — It may now be added that with the appearance of these zones the egg has become a *blastula*, while the entire cellular and partially cellular area, including the zone of junction and the zone of overgrowth, may henceforth be referred to as the *blastoderm* (Fig. 156, *D*). Its establishment terminates the period of segmentation as distinguished from that of gastrulation. Nevertheless, the outward extension of the blastoderm over the yolk continues for some time after the latter process is completed. This is brought about by the steady out-pushing of the zone of overgrowth and the germ wall, which not only themselves increase somewhat in width (particularly the germ wall), but leave behind them an ever-widening area of extra-embryonic ectoderm, mesoderm, and endoderm. The exact method by which these cell layers are differentiated within the extra-embryonic blastoderm will be discussed in detail later.

Before proceeding with a description of gastrulation, and the origin of these layers in the Bird, it is desirable to recall one point discussed in connection with the Fish and Gymnophiona. It may be remembered that in both the latter forms the rim of the blastoderm was homologized with the lip of the blastopore. It was, nevertheless, indicated in the introduction that this homology is denied by some in the case of the Bird because of the method of gastrulation in this form as about to be de-

scribed. This problem will be mentioned again in that connection. One point of functional similarity between the rim of the blastoderm in the Fish and Gymnophiona and that in the Bird is, however, already apparent. The process of overgrowth of the yolk, or epiboly, by the blastodermal rim, call this rim what one will, is the same in all.

G

ASTRULATION¹ AND DEVELOPMENT THROUGH THE FIRST DAY² OF INCUBATION

GASTRULATION

THE problem of gastrulation in the Chick is one which has received considerable attention both by study of normal total blastoderms and sections, and more recently by experimental procedures. The latter have involved removing living blastoderms and parts of blastoderms to artificial locations, cutting them at various levels, and marking them with vital dyes. The object has been to determine exactly what movements are taking place, where the primary layers are derived from, and what parts of the early blastoderm give rise to specific features of the early embryo. In spite of all this study investigators are still not in entire agreement on the answers to some of the above questions. At the risk of satisfying no one, therefore, the writer is going to attempt to piece together a more or less connected account. In doing so it will be necessary to select conclusions regarding some of the moot points from different workers on the basis of what seems to us most reasonable and likely. Statements over which there is especial disagreement will be indicated in order that the student may be aware of what is most generally accepted and what is not. It will be noted at once that the accepted items largely concern the existence of successive stages of certain structures. Those matters under controversy, on the other hand, have mainly to do with the interpretation of these structures, i.e., questions of their homologies, of how they arise and what they produce. The investigators whose accounts have been particularly consulted are Chen, Hunt, Rawles, Rudnick, Woodside, Pasteels, Peter and Spratt. The review of the subject by Rudnick ('44) is especially valuable as a critical summary of the situation to that date, and the interested student is referred to this and to articles by the other authors cited for further details.

¹ Gastrulation is usually only slightly under way when the egg is layed (see below).

² The term day as used in connection with the development of the Chick refers to a period of 24 hours.

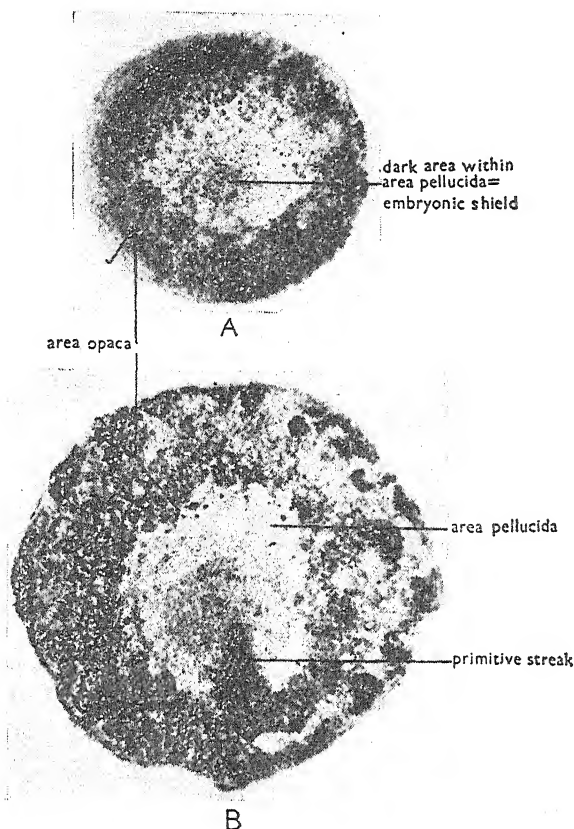


Fig. 158. — Photographic surface views of early Chick blastoderms. After Spratt. *A*. An unincubated blastoderm of the pre-streak stage. The dark area opaca, and the lighter area pellucida with a darker region within it, the embryonic shield, are clearly shown. *B*. A blastoderm of eight hours incubation showing the primitive streak at an early stage. The darker embryonic shield lateral and anterior to the streak shows clearly but is not labelled in this case.

The Area Pellucida and Area Opaca. — As gastrulation begins the blastoderm above the subgerminal cavity becomes thinned somewhat by the outward movement of its cells. For this reason, the absence of adhering yolk and the existence of the cavity, this central region when viewed from above appears different from the surrounding parts. Thus when observed upon the living egg it appears darker, while in a stained blastoderm mounted upon a slide it is more translucent. Be-

cause of this it is referred to as the *area pellucida*. The surrounding parts comprising the zone of junction and zone of overgrowth on the other hand are more whitish in the living egg, and more heavily stained and opaque in preserved material. Therefore this surrounding region is appropriately termed the *area opaca* (Fig. 158).

The Primordial Hypoblast. — The first step in actual gastrulation seems to be the appearance within the subgerminal cavity of a sec-

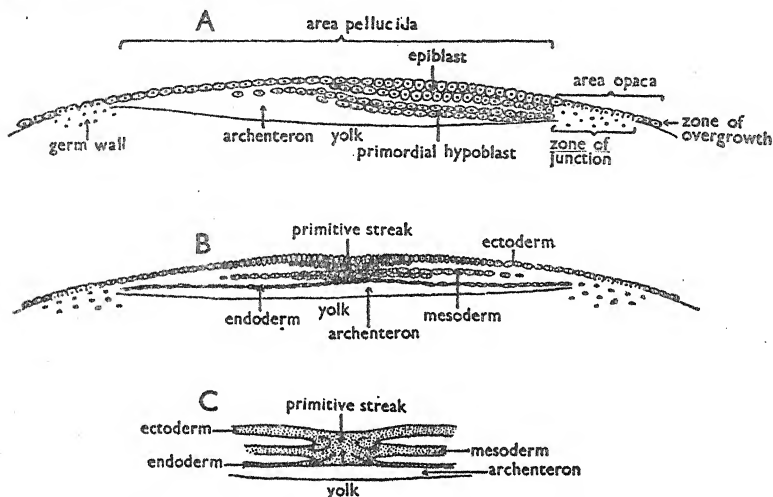


Fig. 159. — Diagrams of sections through the Chick blastoderm showing the origins of the primordial hypoblast, the definitive endoderm and the mesoderm. *A*. A median sagittal section through a very early Chick blastoderm such as is shown in Fig. 158, *A*, in which the primitive streak has scarcely begun to form. The hypoblast has just been delaminated (and, or, infiltrated) from the epiblast. The area opaca at this stage consists only of the zone of junction and the zone of overgrowth. At this stage the zone of junction is mostly, though not entirely, identical with the germ wall. Thus it will be noted that the latter extends slightly medially beneath the archenteric space. Later only a small part of the outer periphery of the germ wall is thus identical with the zone of junction. *B*. A cross section of the blastoderm of a slightly later stage where the streak has formed, and mesoderm, and perhaps definitive endoderm, is arising in connection with it in the manner indicated in *C*.

ond cell layer which may be termed the *primordial hypoblast*. The space between this layer and the underlying yolk then, as in the case of the Fish, becomes the *archenteron*. The new layer is designated "primordial" because it appears doubtful that it represents the final or definitive hypoblast, or at least that it represents all of it. The method of its origin is one of the disputed questions. It was formerly supposed to originate by involution of marginal cells through a temporary interrup-

tion in the zone of junction along a small part of the blastodermal rim. The location of this activity if it occurred would of course represent, as in the Fish, the dorsal blastoporal lip, and hence also as in the Fish the future posterior region of the embryo. It has even been claimed by one observer that an actual invagination occurs here, giving rise to a pocket with both roof and floor, i.e., a complete archenteron (Jacobson, '38). At present, however, the belief in either involution or invagination as defined in this text is no longer entertained in the case of the Chick. Instead Peter ('38) and others seem to think that the process is rather what we have designated as infiltration. That is to say, these workers believe that individual cells wander in from the surface and detach themselves within the subgerminal cavity where they eventually become arranged to form a more or less continuous layer. It should be noted incidentally that the sponsors of this view do not use the term infiltration, preferring to call the inwandering of these individual cells "invagination." This, however, seems to the writer a misnomer and confusing. At all events regardless of the terminology the activity is said to be as designated.

It must further be stated that those who are agreed on the character of the process as described are not entirely agreed on just where it takes place. According to some (Pasteels, '45) it occurs more or less all over the pellucid area of the blastoderm. Peter, however, seems to think it takes place mainly toward the future posterior side, especially near the margin, with a subsequent forward movement. This would approach more nearly the older idea of an involution from one side.

Finally it may be said that some workers (Spratt, '46) describe the process of hypoblast origin as one of splitting off or delamination of cells rather than their inwandering (Fig. 159, A). Also at least one investigator (Fraser, '54) has observed the infiltration of cells from the epiblast at the anterior and posterior borders of the area pellucida, suggesting once more a sort of modified involution at these borders, but without interruption of continuity in the epiblast. It is of interest to note here that a similar problem regarding the nature of hypoblast origin occurs in the Mammal where again some form of infiltration or delamination seems to occur. This matter will be referred to later in the appropriate connection.

After the formation of the layer of primordial hypoblast it might be assumed that gastrulation, as defined in this text, would be complete. However, as noted, this hypoblast is probably only part of the definitive hypoblast (endoderm), and in the Bird more than in the Frog and Fish

it is difficult to separate sharply the origin of the definitive hypoblast from the origins of the mesoderm and notochord. Also the appearance of the primitive streak, a structure previously related primarily to gastrulation, is, as we shall see, probably involved here both in the formation of definitive hypoblast, and in the origin of mesoderm and notochord. We shall therefore have to continue our discussion of these activities more or less simultaneously as a later aspect of gastrulation.

Before proceeding with this it may be remarked that it is at about this stage of development that the egg is usually laid. The diameter of the entire blastoderm is approximately 3.36 mm., and that of the area pellucida about 2.16 mm. (Spratt, '46). If unincubated it may remain in this condition for some time. If incubation ensues before too long an interval has elapsed further development proceeds as follows:

The Primitive Streak.—The second step in gastrulation is the development of the *primitive streak* whose history is as follows: Just before the streak begins to form, about three fourths of the area pellucida, as viewed from the surface, starts to become more darkly staining and opaque toward what later proves to be its posterior side. This is due both to a thickening of the epiblast in this region, and to the presence of the underlying hypoblast. The part so affected is sometimes designated as the embryonic shield, though not entirely homologous with the region similarly named in the Fish as previously described (Fig. 158, *A*). Presently the streak begins to appear at the posterior side of this shield, as a still more darkly staining somewhat triangular structure with its base in contact with the inner rim of the area opaca (Fig. 158, *B*). This appearance is produced by a further thickening of the epiblast in the region concerned in a manner to be indicated below. At first the thickened epiblast reaches only a short distance cephalad, but soon, as its growth is completed, its anterior end occurs at about the middle of the pellucid area. As a result of this increase in length the structure loses its triangular shape, and appears more as a broad band or actual streak with a tapering and rounded anterior end. At the same time sections reveal that from its first appearance the thickened epiblast of this band has been in intimate contact with the underlying hypoblast. A little later the band (primitive streak) becomes still narrower, and a distinct groove develops down its middle with a little twist or irregularity at its cephalic extremity where the groove terminates in a slight pit. The groove is termed the *primitive groove*,³ and

³ The term primitive streak is sometimes rather carelessly used to refer to both streak and groove.

the pit is the *primitive pit*. The latter together with the surrounding cells is called *Hensen's knot* or *Hensen's node*, also the *primitive node* (Figs. 160, 161, 162). The sides of the groove are sometimes designated as the *primitive folds*, having nothing to do of course with the later neural folds. So far as the writer is aware no one questions the existence of these structures as described. Again the real problem concerns the homology of the streak or groove, its origin and its functional relation to the parts about it. Since the answer to the first of these queries depends

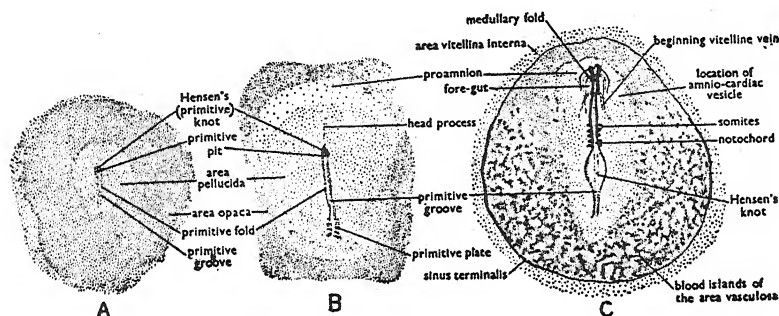


Fig. 160. — Surface of the Chick blastoderm and early embryo. *A*. A pre-incubation blastoderm showing the primitive streak, actually the primitive groove. *B*. An 18 hour blastoderm showing the beginning of the head process (notochord). *C*. A 24 hour blastoderm with embryo well started and the area vasculosa forming.

largely upon the answers to the last two, we shall take these latter up in order. We shall then be prepared to return to the problem of homology.

The Origin of the Streak. — As a result of numerous marking experiments it appears to be fairly clear that the streak originates by the convergence of epiblast cells from the lateral regions toward the place where the initial short "streak" is first seen (Chen, '32, Spratt, '46), (Fig. 163). This produces an aggregation of material here which constitutes the thickening described as characteristic of this structure. It should also be noted, as Spratt points out, that the cells thus aggregated do not pile up upon the surface of the blastoderm, but pass inward, as he expresses it by "invagination." It is this process which almost at once, as previously indicated, brings them in contact with the underlying hypoblast. After being started in this manner the lengthening of the streak occurs, according to Spratt, by the proliferation of its cells as follows: At its front end these cells are so added as always to be at or near the tip, as in the growing point of a plant. Posteriorly the growth seems to be more by intussusception pushing this end back-

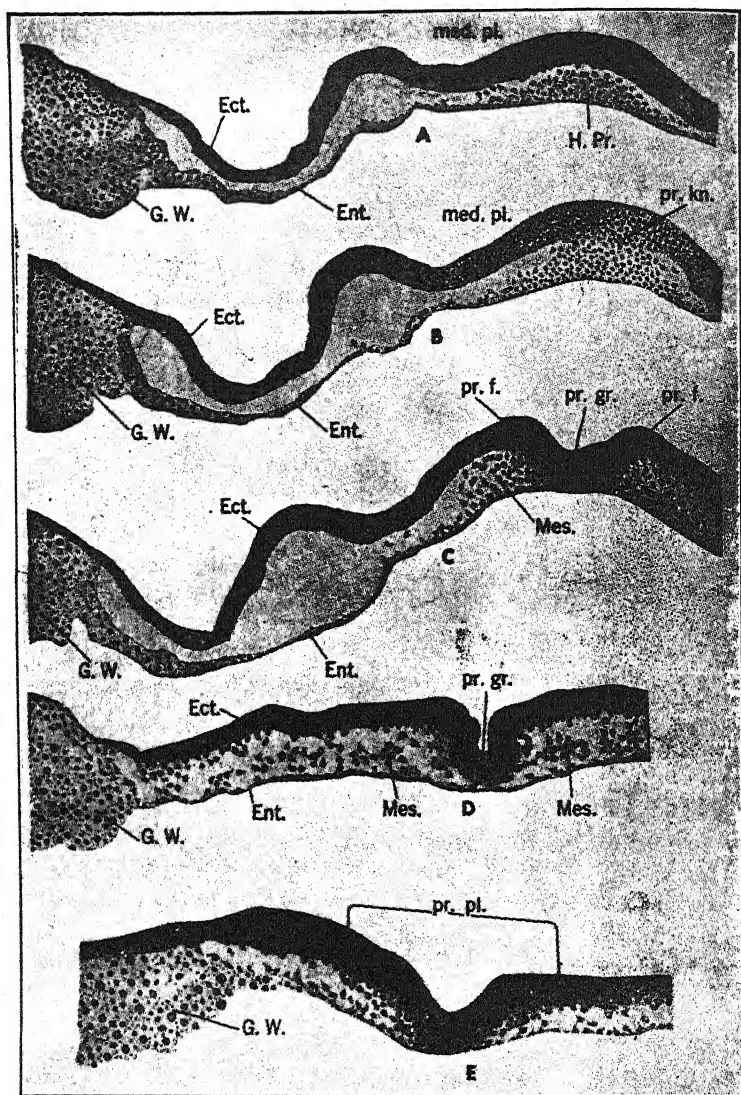


Fig. 161. — Five transverse sections through the head process and primitive streak of a Chick embryo. The head process is very short. From Lillie (*Development of the Chick*).

A. Through the head process, now fused to the entoderm. B. Through the primitive knot. C. Through the anterior end of the primitive groove. D. A little behind the center of the primitive streak. E. Through the primitive plate. The total number of sections through the head process and primitive streak of this series is 102. B is 4 sections behind A. C is 12 sections behind A. D is 59 sections behind A. E is 87 sections behind A.

Ect. Ectoderm. Ent. Entoderm. G.W. Germ wall. H.Pr. Head Process. med.pl. Medullary plate. Mes. Mesoblast. pr.f. Primitive fold. pr.gr. Primitive groove. pr.kn. Primitive knot. pr.pl. Primitive plate.

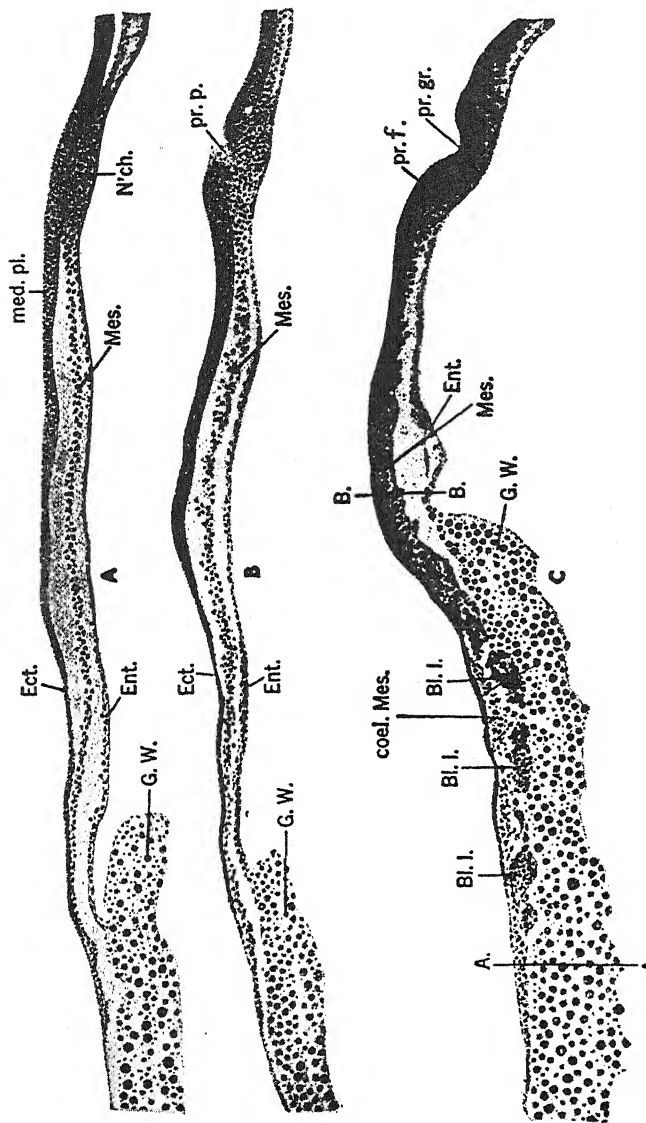


Fig. 162. — Three transverse sections of a late stage, through the head process and primitive streak of a Chick embryo. From Lillie (*Development of the Chick*). *A.* Near the hind end of the head process. *B.* Through the primitive pit. *C.* A short distance behind the center of the primitive streak.

Bl. I. Blood island. *coel. Mes.* Coelomic mesoblast. *Ect.* Ectoderm. *Ent.* Entoderm. *G.W.* Germ-wall. *med. pl.* Medullary plate. *Mes.* Mesoderm in area pellucida. *N.ch.* Notochord. *pr. f.* Primitive fold. *pr. gr.* Primitive groove. *pr. p.* Primitive pit.

ward. Accompanying, and perhaps partially caused by this movement, the whole pellucid area changes its shape from that of a circle to a pear with the small end posterior. Finally it may be stated that this growth of the primitive streak appears to be induced by the underlying primordial hypoblast. This is concluded from the fact that this hypoblast is at

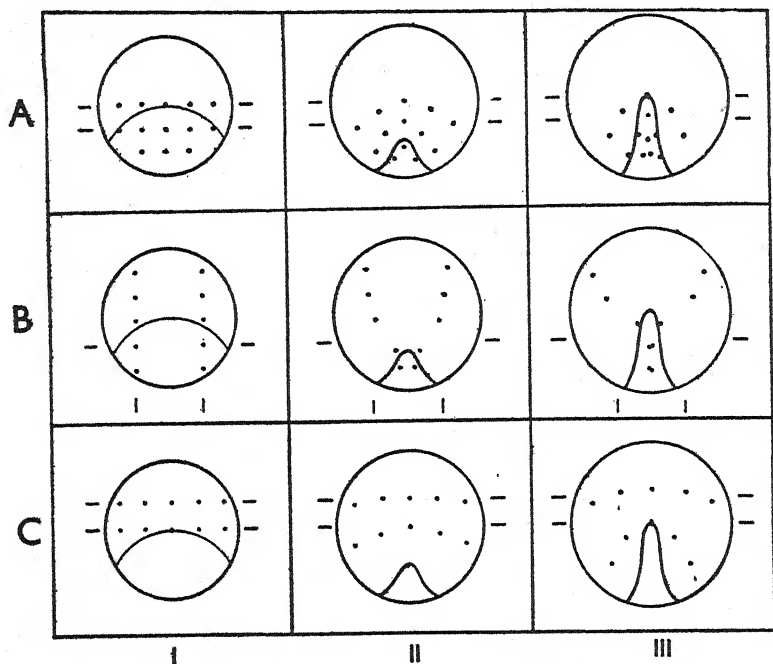


Fig. 163. — A diagram to illustrate the movements occurring on a Chick blastoderm during gastrulation and primitive streak formation. After Spratt. The movements are indicated by changes in the positions of carbon particles placed on the blastoderm at the start of the process. Horizontal rows A, B and C illustrate three different plans of placing the particles. Vertical rows I, II and III indicate the positions of the particles in each plan during successive stages in gastrulation. The short horizontal lines outside the blastoderms are points of reference. Note the general tendency of convergence toward the forming streak.

first chiefly toward the posterior of the blastoderm, and as it spreads anteriorly the growth of the primitive streak follows it. There are also other facts which support this hypothesis (Fig. 164).

Functional Relations of the Primitive Streak.

Differentiation of Mesoderm, Endoderm and Ectoderm. — It is now rather generally conceded that not only are materials moved into the

streak from the outlying epiblast, but they also pass through it to specific destinations (Hunt, '37, Spratt, '46). One of these is apparently a layer of cells pushing out on either side of the streak between the epiblast and the primordial hypoblast. This layer is the *mesoderm*. It is also claimed that some of the cells moving through the streak pass into and augment the previously existing primordial hypoblast (Hunt, '37), (Fig. 159, B, C). Thus this latter layer is converted into *definitive hypoblast*, or as it may now be called *endoderm*. The question as to just how much of the endoderm owes its origin to this movement of cells through

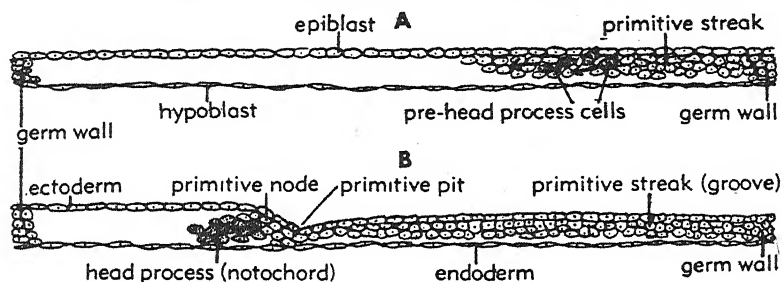


Fig. 164. — A diagram of a median sagittal section through the primitive streak, A, and groove, B, and parts anterior to each, showing the origin of the head process (notochord) according to Spratt and Fraser.

the streak, and how much to the spread of the primordial hypoblast is one of the unanswered questions. As usual after the origin of these layers the remaining epiblast may be called *ectoderm*.

Lastly, it may be noted that the process just indicated in connection with the origin of the mesoderm and endoderm is again what we should term a kind of infiltration. Nevertheless, as will be pointed out subsequently, it does bear some resemblance to the passage of cells around a blastoporal lip, i.e., involution, and might help to account for the development of the groove. Also, as in the case of the inwandering of cells from the surface into the primordial hypoblast, it has been referred to, ambiguously the writer thinks, as "invagination."

The Head Process (Notochord). — This leaves the origin of the notochord still to be accounted for. Accompanying the above-mentioned activities there also appears in front of the primitive streak or groove another somewhat narrower line temporarily termed the *head process* (Fig. 160, B). It begins at Hensen's knot with which it maintains constant contact, and extends anteriorly. Sections reveal that it consists of a line of cells somewhat like the streak, but in this case they have no definite connection with the epiblast, now ectoderm, save at Hensen's

knot (Figs. 161, A; 162, A). This head process rapidly increases in length, and eventually undergoes histological changes to become the *notochord*. Concerning the above statements there is no question. The problem again arises, however, as to where the head process (notochord) originates from, and by what method it develops. It has been claimed that it arises by a splitting off of streak material from the epiblast in a posterior direction. Thus as the head process grows at its back end the streak would shorten proportionally at the front end (Lillie, '19). The streak does indeed shorten, but not proportionally. Hence it has been claimed by others that the head process grows from cells budded off from the anterior end of the streak, and pushed forward.

Finally according to Spratt, '47, and Fraser, '54, the following occurs: At first the streak, as noted, is quite short. As its substance grows anteriorly beneath the epiblast, the cells of the latter, originally just in front of the streak, come to lie posterior to its anterior tip, i.e., somewhat behind the primitive node and pit. Some of these cells then pass into the substance of the streak and forward within it to a point under the node. Here they form a mass from which the head process is budded, almost entirely posteriorly (Fig. 164, A). This means that the primitive streak is forced to recede before it. However, according to Spratt's evidence it does not shorten at its anterior end in the region where it is in contact with the head process. Instead the substance of the streak is "pushed" back, or at least it migrates backward. But though the streak does not shorten at the front end, it does shorten at the back end. It does this simply by "dissolution" into the ectoderm and mesoderm of this region. As indicated in connection with one of the other theories, however, this shortening is not quite at the same rate (i.e., proportional to) the lengthening of the head process. Therefore Spratt suggests that there must be some condensation of material in the shortened streak. Eventually, nevertheless, the latter does entirely disappear, except in so far as its remains may constitute the "end bud" (posterior tip) of the embryo. Figures 164 and 165 illustrate diagrammatically the processes supposed to be involved. This theory of head process (notochord) origin is supported by extremely careful studies based on a somewhat new technique. Instead of the dyes previously used for marking points on the living blastoderm, carbon particles were introduced into it, thereby eliminating the spreading of the marks by mere diffusion. Their movements were then kept track of in relation to certain fixed points outside the area where the critical changes were occurring. The results seem conclusive, but will of course have to be confirmed by other workers.

Distribution of Formative Materials in the Streak and Pre-streak Blastoderm.—In our consideration of gastrulation in the Frog emphasis was laid on experiments indicating the distribution of germ layer materials previous to the gastrulation process. The question naturally arises therefore as to whether it has been possible to make comparable pre-gastrular maps in the case of the Bird. The answer is

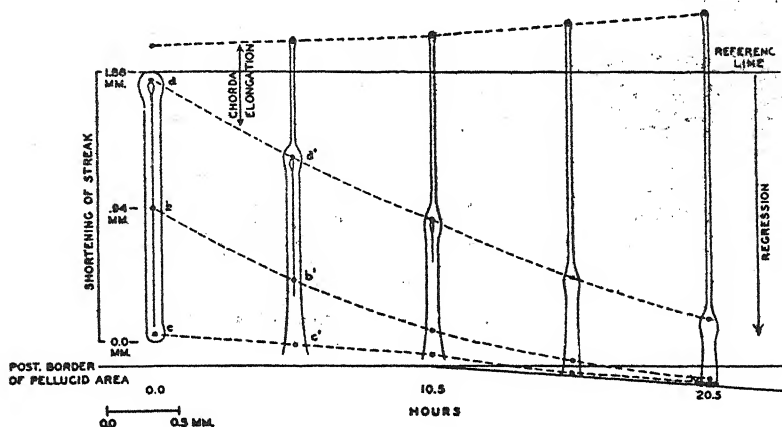


Fig. 165. — A diagram to illustrate the movements occurring in the primitive groove ("streak") and parts connected with it during head process ("chorda") formation. After Spratt. Three cells in the groove were marked by carbon particles just before the head process started to appear as shown by the dots on the streak at the left. As the head process forms, the location of the particles and the changes in the parts are seen in successive stages as one passes to the right. Note what happens to the groove as the head process lengthens.

that if one considers the existence of the primordial hypoblast as denoting the completion of gastrulation, such maps have not been made. This is not surprising since this stage is reached prior to the laying of the egg. However, in so far as the formation of the primitive streak is regarded as part of gastrulation, the answer is quite otherwise. Many studies have been made of the potentialities of the various regions of the blastoderm beginning with the late pre-streak stage, and extending on to that of the head process. Wetzel, '29, Rawles, '36, Pasteels, '37, Rudnick, '38, most recently Spratt, '42, and others have worked on this problem largely by two techniques. (1) They have vitally stained or otherwise marked the various regions of the blastoderm in situ, and noted the subsequent movements of the stained parts. (2) They have isolated pieces of the blastoderm on various culture media, and observed what each piece is able to produce. Naturally, the later in de-

velopment the experiments were performed, the more precise have been the results, but also of course the further they are removed from the pre-gastrular situation. It is not feasible to go very deeply into this topic,

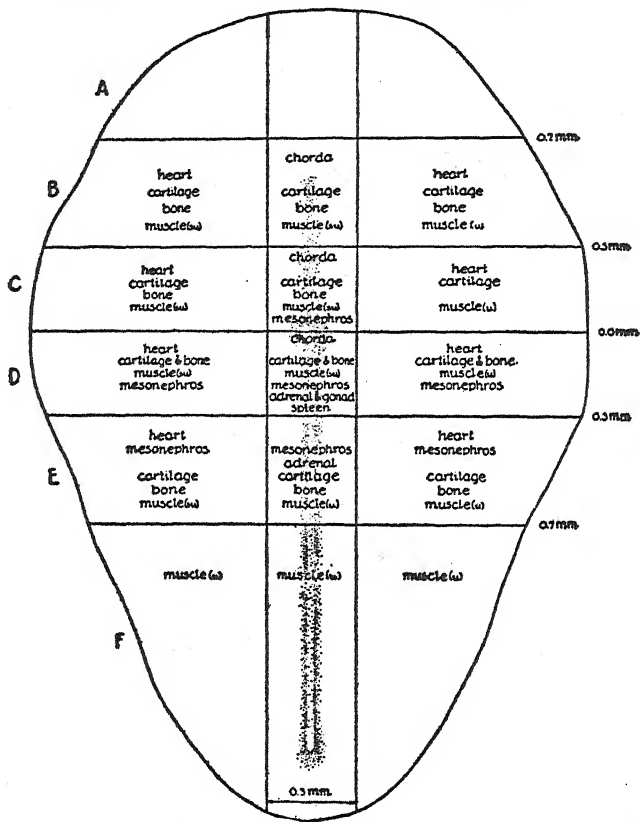


Fig. 166. — A diagram showing the sections into which a primitive groove and head process stage of a Chick blastoderm was cut, and the tissues and structures derived from the mesoderm of each isolated piece. After Rawles.

but we may present as an example of the conclusions of some of the work on later stages one of the maps by Rawles (Fig. 166). With reference to this map it should be stated that the results upon which it is based were all obtained by the isolation method, and it must be admitted that this method has one weakness. Since the isolate is in a new environment the potentialities which it exhibits are not necessarily those

it would have realized had it been left intact. In fact they are apt to be greater, due perhaps to the removal of inhibition by neighboring parts, or to lack of specific induction by those parts. It should be understood that though the map selected is for mesoderm only this does not mean that this was the only layer studied, or that the layers were transplanted separately. The results for the different layers were merely recorded separately as a matter of convenience, and our choice of the map of this particular layer has no special significance. As regards the conclusions, in view of the results on earlier stages to be indicated presently, it is perhaps noteworthy that for all layers the regions capable of producing the most structures were those near the center of the blastoderm, i.e., about Hensen's node. It is of further interest that the left side showed more potentialities than the right.

An example of a study of very early stages (early streak and late pre-streak) is that of Spratt's isolation work ('42). Stated very briefly his conclusions are essentially as follows: He finds, in substantial agreement with most others, that prospective neural plate material lies near the center of the area pellucida. Notochord, on the other hand, is formed from the region just behind this in about the third quarter of the pellucid area. Potential mesoderm, including heart forming material, appears to be somewhat more widely diffused both anteriorly and posteriorly. From this we see that although it has not been possible to map prospective germ layer and organ-forming regions quite as early or as accurately as in the case of the Amphibians, some progress has been made. Thus it is at least evident that the materials for the nervous system, the mesoderm and notochord exist independently in more or less separate, though overlapping, localities at the pre-streak stage, and that they are subsequently moved into their definitive positions as the streak develops. Whether the separation of these substances occurs still earlier, perhaps even in the unsegmented egg, as in *Amphioxus* and the *Amphibia*, we do not yet know.

THE HOMOLOGY OF THE PRIMITIVE STREAK

It will be recalled that the term primitive streak was used in connection with the Frog, Fish and *Gymnophiona* to denote the line formed by the closed blastopore. The question now is whether the primitive streak of the Chick is really homologous with this line, and hence represents a closed blastopore.

REASONS AGAINST HOMOLGY OF PRIMITIVE STREAK AND CLOSED BLASTOPORE

- (1) The streak is not at any time an opening into the archenteron, as a real blastopore is supposed to be.
- (2) The origin of the primordial hypoblast at least is not related to it, nor to its "lips" (sides of the groove).

REASONS FAVORING HOMOLGY OF PRIMITIVE STREAK AND CLOSED BLASTOPORE

(1) In the Frog and Fish it was shown that there is a convergence of materials on the outside of the blastula toward the forming blastopore. Various marking experiments on the epiblast of the Chick blastoderm show similar movements of material in its postero-lateral regions toward the forming primitive streak.

(2) In *Amphioxus*, the Frog, and Fish there was shown to be an involution of the materials just mentioned over the dorsal lip into the roof and sides of the archenteron. In the Chick there is, strictly speaking, no blastopore in the region of the streak, and hence no blastoporal lip. The streak, however, does have contact with the primordial hypoblast, and it does develop along either side of it, ridges which would correspond to the lateral lips of a blastopore. Most important of all it has been shown that there is a movement of material through these ridges into the forming mesoderm, and possibly into the endoderm. In other words as previously suggested there is a kind of "involution," in which the presumed homologues of the blastoporal lips are intimately involved.

(3) In *Amphioxus*, the Frog, and Fish the notochord arises from material involuted at the dorsal lip of the blastopore, and budded forward from that region. In the Chick we have seen that the notochord originates from cells passing inward not, to be sure, through the pit, whose anterior rim is the homologue of the dorsal blastoporal lip, but posterior to it. Yet even here such movement is suggestive, even though the material grows backward instead of forward to form the notochord.

(4) In *Amphioxus* and the Frog we have found the neurenteric canal originating by the uniting of the neural folds over the anterior part of the closing blastopore (primitive streak), while in the Fish Kupffer's vesicle, the homologue of that canal, occurs at the same location. Now in the Chick, to be sure, there is no neurenteric canal at the anterior end of the primitive streak. There is, however, a pit at this point which is

eventually covered by the neural folds, and in some Birds (Duck, Goose and others) this pit does finally open to the archenteron. Thus in these cases a neurenteric canal, incipient or actual, is formed in the proper place if the streak be regarded as a closed blastopore.

(5) In the Frog, certainly, and probably in the Fish, the anus forms at the end of the closed blastopore opposite from the neurenteric canal, the line between the two being designated as the primitive streak. We have just seen that at least in some Birds what amounts to a neurenteric canal forms at the anterior end of the streak. On this basis the anus should arise at the posterior end of this structure, and apparently it does so (Lillie, '19).

(6) In the Frog the material in and about the lip of the early blastopore is known to have remarkable inductive powers. In the Chick the primitive streak is said by some (Woodside, '37) to have similar powers when transplanted beneath the epiblast of a very early primitive streak host.

EXTENSION OF THE GERM LAYERS AND FORMATION OF THE AREAS VASCULOSA AND VITELLINA

Up to this point the processes of gastrulation and germ layer formation have been considered only in relation to the area pellucida. It now remains to consider what is happening in these connections in the area opaca.

ORIGIN OF ENDODERM IN THE AREA OPACA

In connection with the origin of the primordial hypoblast before the advent of the primitive streak, it was noted that this hypoblast arose by the inwandering (infiltration) of cells from the surface of the blastoderm, or by delamination from its under-surface. It was also said that this probably occurs mostly about the posterior half of the blastoderm, perhaps more especially around its margins. This hypoblast was then supposed to be later augmented to form endoderm by infiltration of cells through the streak. Upon this basis it is not surprising therefore to learn that according to some accounts the endoderm of the area opaca is derived as follows:

It is said that the nuclei from the zone of junction keep moving in toward the area pellucida. As they do so, the cytoplasm about each nucleus engulfs yolk granules, and becomes cut off from that about it to form a definite cell. Thus the lower part of the germ wall becomes or-

ganized so that toward its inner margin (the edge of the area pellucida), it begins to form a cell layer. This layer is endoderm which becomes continuous with the definitive endoderm of the area pellucida. If this account be correct it would seem that a process which is essentially infiltration, in this case from the margins of the blastoderm, is still giving rise to some of the endoderm, i.e., that of the area opaca. It may now be stated that because of its subsequent history the endoderm of this area is often referred to as *yolk-sac endoderm*.

THE BLOOD ISLANDS AND THE MESODERM IN THE AREA OPACA

The Blood Islands. — Though the origin of the endoderm of the area opaca has been described first, it actually follows slightly, both in time and peripheral location, the formation of the mesoderm which comes about somewhat indirectly as follows: It appears that cells from the postero-lateral margins of the mesoderm in the area pellucida wander into the upper part of the germ wall of the area opaca, where they also engulf yolk granules. These cells become aggregated into small masses in this region, and these masses presently anastomose to form a network. Throughout this network spaces or lacunae are then developed which contain little groups of cells. Presently the walls of the lacunae become differentiated into the flat endothelial cells characteristic of the inner lining of blood vessels, while the cells within the lacunae become blood corpuscles. Because of the manner of their formation these corpuscles are at first necessarily aggregated into groups, which appear from the surface as darker splotches. These splotches of corpuscles, or forming corpuscles and their surrounding endothelium, are known as *blood islands*. Obviously they arise somewhat previous to the main parts of the circulatory system with which they presently become connected (see below).

The Mesoderm of the Area Opaca. — Coming now to the mesoderm of this region we find that it is produced by the budding off of cells from the surface of the developing blood islands, between the islands and the overlying ectoderm. At its inner margin this mesoderm like the endoderm becomes continuous with that occurring in the area pellucida (Fig. 162, C).

It remains to state that because of the indirect method of production of this mesoderm its source as just described has been questioned by some. Thus it has been claimed that the blood islands, and hence the mesoderm, come from cells originating in the zone of junction in the

same manner as the endoderm of this area. The account as we have previously given it, however, is afforded strong support by the following fact: Patterson ('09) has shown that where the mesoderm of the pellucid area fails to reach the germ wall no blood islands and no mesoderm develop in the area opaca. It may finally be noted that if the mesoderm of this area does arise from that in the area pellucida, as seems most probable, then like the latter it also, though somewhat indirectly, has its ultimate source in the primitive streak.

Though beginning in the postero-lateral regions as indicated the processes thus described are gradually working forward upon each side of the area opaca, the proliferated mesoderm of the area pellucida keeping pace with that which arises from the blood islands further out. Finally, as the level of the anterior end of the head process is reached, the mesoderm of the pellucid area ceases to form. That in the area opaca, however, continues upon either side as a pair of anteriorly projecting wings, which after proceeding somewhat beyond the future head region begin to turn toward one another so that they eventually meet (see second day). In the area pellucida, however, immediately in front of and slightly to the sides of the head region, no mesoderm forms for some time, the zone thus marked out being termed the *proamnion* (Fig. 160, C). Following the advent of the blood islands it soon becomes possible to subdivide the blastoderm into further parts as follows:

The Area Vasculosa. — The blood vessels, having once become formed in the area opaca, are not confined there. Very soon, especially postero-laterally, they begin to extend into the area pellucida, where they unite with other vessels which have arisen *in situ* from the mesoderm; the entire region thus covered by them is then termed the *area vasculosa*. Presently, around the outer edge of this area, its boundary begins to be clearly defined by an encircling blood vessel, the *sinus terminalis* (Fig. 160, C).

The Area Vitellina. — The remainder of the blastoderm peripheral to the area vasculosa is termed the *area vitellina*, and is in turn subdivided as follows: The part at and near the blastodermal rim continues to consist of the relatively narrow zone of overgrowth and zone of junction, and is known as the *area vitellina externa*. Between this area and the area vasculosa there is then a region which, with continued expansion of the blastoderm, soon becomes rather extensive. Within it, although the germ wall is becoming occupied with yolk filled cells, these cells have not yet become definitely organized into endoderm or blood islands. Nevertheless this part of the wall is clearly separated from the

epiblast above it, and is beginning to be more or less delimited from the non-cellular yolk beneath it. The relatively broad region thus characterized is called the *area vitellina interna* (Figs. 167, 170, *A*, *E*).

As has already been suggested, all of these areas, while retaining the same relative position as regards each other, are constantly moving outward over the surface of the yolk by a process of epiboly (Fig. 167).

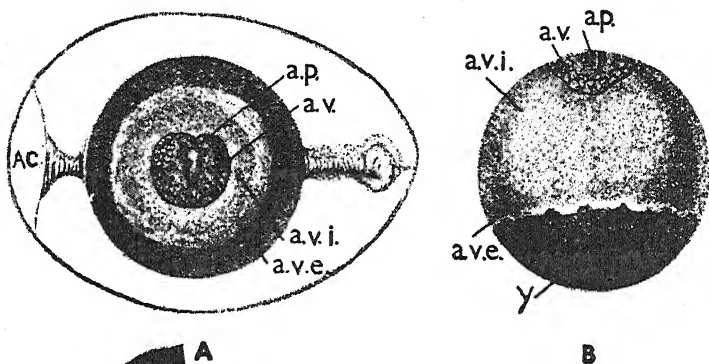


Fig. 167. — *A*. Hen's egg at about the twenty-sixth hour of incubation, to show the zones of the blastoderm and the orientation of the embryo with reference to the axis of the shell. *B*. Yolk of hen's egg incubated about 50 hours to show the extent of overgrowth of the blastoderm. From Lillie (*Development of the Chick*). After Duval.

a.c. Air chamber. *a.p.* Area pellucida. *a.v.* Area vasculosa. *a.v.e.* Area vitellina externa. *a.v.i.* Area vitellina interna. *Y*. Uncovered portion of yolk; i.e., the "yolk blastopore" or yolk-sac umbilicus (see below, and page 362).

FURTHER HOMOLOGIES

The Margin of the Blastoderm. — It was stated in connection with the Fish that the margin of the blastoderm, or germ ring in that form was entirely homologous with the blastoporal lips, and that it finally closed to form a primitive streak. It was then indicated that in the Gymnophiona the margin of the blastopore is again the homologue of the blastoporal lips. In this instance, however, these lips (germ ring) become divided into two parts by the early contact of points on the lateral lips a short distance from the dorsal lip. In this manner a small true blastopore (later a primitive streak) is formed immediately in front of which the embryonic axis proceeds to develop. The remainder of the blastodermal rim is then employed in covering the yolk. As it completes this process there appears what amounts to a second or yolk-sac blastopore, with the closure of which the yolk is entirely enveloped.

The question now to be answered is what if any homologies exist between the avian primitive streak and blastodermal rim, and the blastopores of the Fish and Gymnophiona. We have already given reasons for homologizing the primitive streak of the Chick with the streak of less advanced forms such as the Fish and Frog in which this structure represents the entire closed blastopore. What then of the remaining blastodermal rim in the Bird?

In answering this let us first consider the character, and then the behavior of this rim. From what has been said it is clear that according to present views there is no real involution at the blastodermal rim of the Chick. Hence the epiblast and primordial hypoblast do not actually unite along this line as at the typical lip of a blastopore. This is most clearly true in the very early stages when the infiltration or the delamination of primordial hypoblast cells is said to occur more or less all over the blastoderm. Even at this time, however, there is some evidence that this process is more active about the postero-lateral margins. Later, moreover, when the area vitellina externa has been established it has been indicated that the origin of the cells for the endoderm of the yolk sac, according to many, is mainly dependent upon nuclei migrating from the zone of junction. Thus it can be said that a kind of modified involution is after all occurring at essentially the margin of the blastoderm, and that ectoderm and endoderm are ultimately in contact in that region. So much for the character of the margin. As to its behavior, it has already been said that the blastoderm spreads over the yolk by the usual process of epiboly, and this continues until finally the yolk is completely enveloped. By virtue of its method of formation the covering thus developed consists of all three germ layers, and is called the *yolk-sac*.

Upon the basis of both structure and function, therefore, it is evident that the blastodermal rim of the Chick bears a striking resemblance to the blastoporal lips or germ ring of the Fish, and even more to that of the Gymnophiona. Indeed there are only two essential differences between the rim of the blastoderm in the latter and that in the Bird. One is the fact that in the Gymnophiona there is definite involution at one point on the margin, while in the Bird there is not. The second difference is that in the Gymnophiona the blastoporal lips (blastodermal rim) immediately adjacent to the region of involution soon fuse to form a primitive streak. In the Bird, on the other hand, the primitive streak is apparently formed by a convergence of material in the posterior part of the blastoderm, but not from material actually in the blastodermal

rim. In both cases the remainder of the yolk beyond the blastoderm is temporarily uncovered, constituting the so-called *yolk-sac blastopore* (Fig. 168). This is later enclosed by a yolk-sac in the Bird, and by what virtually amounts to that in the Gymnophiona. In the Fish, of course, the blastodermal rim is not thus divided into two parts, and hence there is no question about the homology of all of it with a blastoporal lip. In the Fish, however, there is no endoderm in the yolk-sac.

Summary of Gastrulation Processes and Homologies in the Chick.—We may conclude the discussion of gastrulation by summarizing the processes involved in the Chick as follows: According to

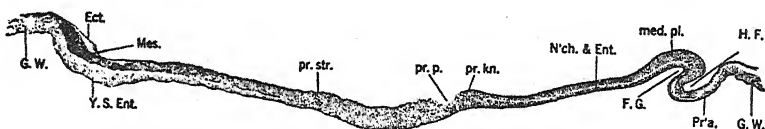


Fig. 168. — Median sagittal section. Stage of the first intersomitic groove. (Cf. Fig. 169). Owing to the bending of the primitive streak the section passes to one side of the middle line posteriorly. From Lillie (*Development of the Chick*).

Ect. Ectoderm. F.G. Fore-gut. G.W. Germ-wall. H.F. Head-fold. med.pl. Anterior end of medullary plate. Mes. Mesoderm. N'ch + Ent. Notochord and entoderm. Pr.a. Proamnion. pr.kn. Primitive knot. pr.p. Primitive pit. pr.str. Primitive streak. Y.S. Ent. Yolk-sac entoderm.

the definitions adopted in this book they would include infiltration (i.e., a modified kind of involution), or (and) delamination, convergence and epiboly.

As to homologies, the primitive streak of the Bird is probably homologous with all other primitive streaks, including those in the Frog, Fish, Gymnophiona, and, as we shall see, the Mammal. Furthermore, there is good reason to homologize the blastodermal rim plus the primitive streak of the Bird with the whole blastodermal rim of the Fish, though the latter contains no endoderm. Likewise we may equally well homologize the rim of the blastoderm of the Bird minus the primitive streak with the rim minus the streak in the Gymnophiona.

DETERMINATION OF THE EMBRYONIC AXIS

It is of course obvious that whatever fixes the position of the primitive streak determines the embryonic axis. The question therefore is what fixes the position of the streak. We must immediately answer that, as in the case of the Fish, we do not certainly know. However, there are some reasonable hypotheses up to a certain point.

If a hen's egg is allowed to rest on its side for a short time it will be

found upon opening it that the yolk (ovum proper) has turned so that the blastoderm is uppermost. Furthermore, if the egg is fertile, and has been incubated, the long axis of the primitive streak, and hence of the embryo, is sometimes exactly, but more often roughly, at right angles to that of the egg shell. Lastly, it will also be true that if the small end of the shell is to the right of the observer, the anterior end of the streak, and hence later the head end of the embryo, will usually be away from him (Fig. 167). These facts have long been known, but in themselves only raise further questions, to wit: Why is the embryo transverse to the length of the shell? Why is the head end away from the observer and why are there exceptions? These are the crucial points. It may be stated to begin with that, granted one initial

assumption, one group of known facts might account for the transverse position, the direction of the head and the exceptions. The unproved assumption and the facts are as follows:

The assumption is that the egg passes from the ovary into the oviduct in such a position that the blastoderm will rest against the wall of the duct, not toward its lumen. It has been suggested by T. H. Morgan ('27) that this might occur if the ovum is regularly more compressible in any axis at right angles to the one vertical to the blastoderm. Granted this initial assumption, it is then known that the blastoderm retains its position against the side of the duct as the ovum passes along it, revolving

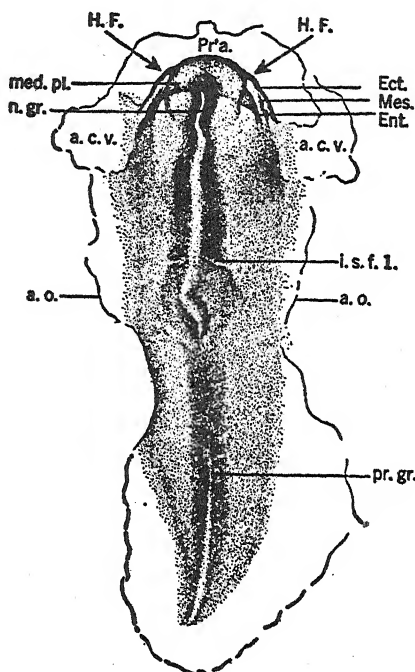


Fig. 169. — Stage of first intersomitic groove drawn from an entire mount in balsam by transmitted light. From Lillie (*Development of the Chick*).

a.c.v. Amnio-cardiac vesicle. *a.o.* Inner margin of Area opaca. *Ect.* Ectoderm. *Ent.* Entoderm. *H. F.* Head-fold. *i.s.f.l.* First intersomitic furrow. *med.pl.* Anterior end of medullary plate. *Mes.* Mesoderm. *n.gr.* Neural groove. *pr.gr.* Primitive groove. *Pr.a.* Proamnion.

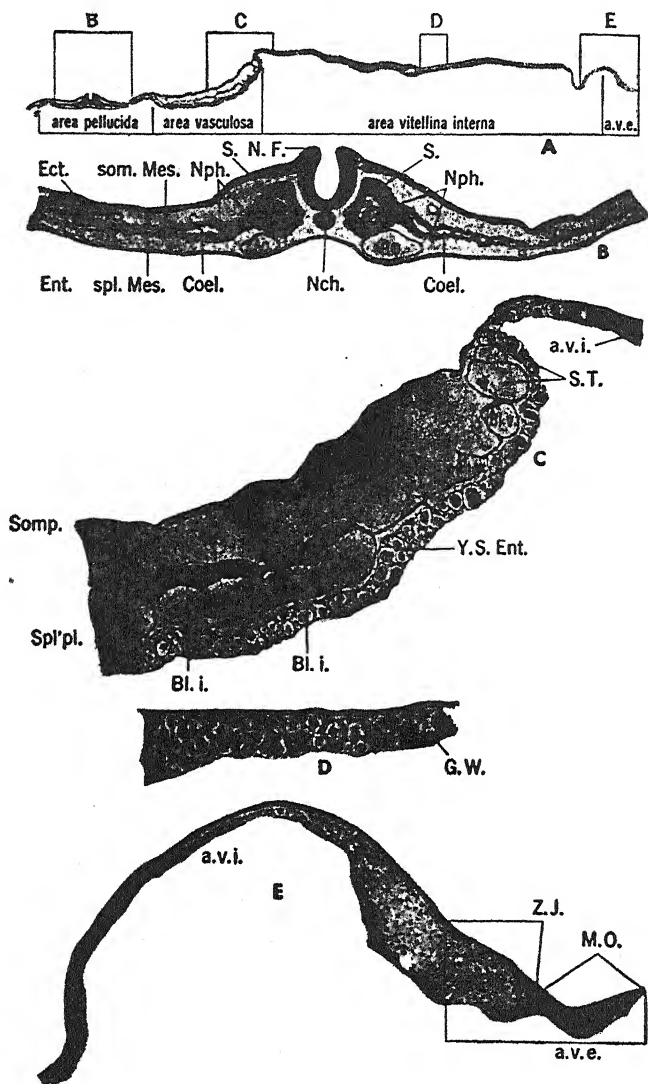


Fig. 170. — A. Transverse section across the axis of the embryo and the entire blastoderm of one side. The section passes through the sixth somite of a 10s embryo, and is intended to show the topography of the blastoderm. The regions B, C, D, E are represented under higher magnification in the Figs. B, C, D, E. From Lillie (*Development of the Chick*).

ao. Dorsal aorta. *a.v.e.* Area vitellina externa. *a.v.i.* Area vitellina interna. *Bl.i.* Blood island. *Bl.v.* Blood vessel. *Coel.* Coelom. *G.W.* Germ wall. *M.O.* Margin of overgrowth. *Nch.* Notochord. *N.F.* Neural fold. *Nph.* Nephrotome. *S.* Somite. *Somp.* Somatopleure. *Spl'pl.* Splanchnopleure. *Som.Mes.* Somatic layer of mesoblast. *spl. Mes.* Splanchnic layer of the mesoblast. *S.T.* Sinus terminalis. *Y.S.Ent.* Yolk-sac entoderm. *Z.J.* Zone of junction.

as it goes. This means that the blastoderm traces an imaginary spiral path around the wall of the duct. It is also known that the small end of the shell is usually found at the leading end. Under such circumstances Morgan further points out that the following conditions might then ensue. As the egg revolves, the two sides of the blastoderm might be under unequal pressure. This might then determine the transverse position

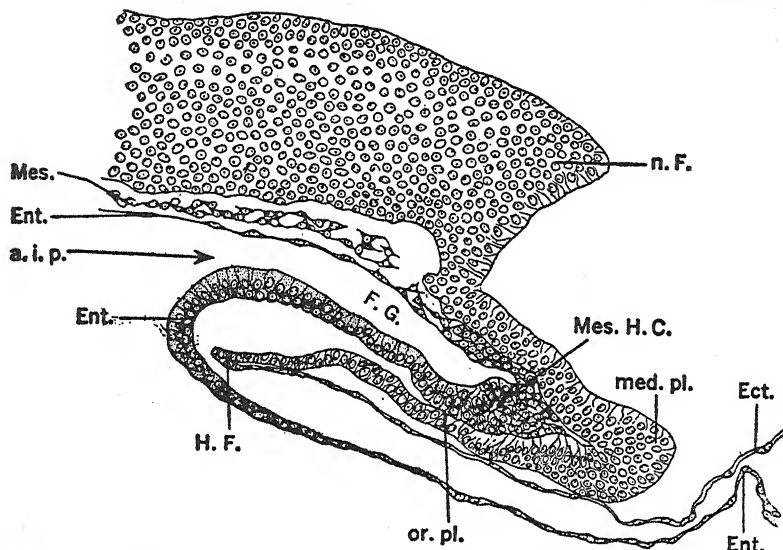


Fig. 171. — Median longitudinal section of the head, stage of 4 s. The section passes through the length of one of the neural folds just behind the anterior end. From Lillie (*Development of the Chick*).

a.i.p. Anterior intestinal portal. Ect. Ectoderm. Ent. Entoderm. F.G. Fore-gut. H.F. Head-fold. Mes. Mesoderm. Mes.H.C. Mesoblastic head cavity. n.F. Neural fold. or.pl. Oral plate.

of the primitive streak, its long axis lying parallel to the direction of pressure. Furthermore, the pressure might presumably be greater on the side toward which the egg was revolving. If so, and if the egg always revolves in the same direction, this might determine that the anterior end of the streak and embryo would always be on a certain side. Bartelmez ('18) has added the notion that the primitive streak axis is determined before the egg leaves the ovary. Then, if as suggested, it always passes into the duct in a certain way this might result in making the primitive streak axis always transverse to the duct and shell. The assumption of Bartelmez may be true, but there is no adequate proof for it, and it seems only to push the ultimate solution further back.

Morgan's theory involves fewer unproved premises, and, due to slight differences in direction of pressure, may account for the variations.

THE HEAD FOLD

A short distance in front of the anterior end of the head process, there develops shortly a slight depression, and immediately posterior to this depression a crescentic fold appears, involving both ectoderm and endoderm (Figs. 168, 169, 171). Its ends extend almost from one side of the area pellucida to the other. The crest of this fold is not raised perpendicularly to the surface, but extends forward so that it overhangs the depression indicated above. It is the *head fold*, and its anterior edge marks the anterior end of the embryo. The lateral and posterior limits of the embryo are not distinguishable until much later.

THE FORE-GUT

From the method of its formation, the cavity within the head fold is necessarily lined by endoderm which is co-extensive with the endoderm of the archenteric cavity posterior to it. It is the anterior portion of the future *fore-gut*, the portion which may be said to represent the pharyngeal region. It is a broad, flattened cavity, and as suggested, opens posteriorly into the extensive archenteric space over-lying the yolk. The region of this wide opening is known as the *anterior intestinal portal*. The endoderm on the antero-ventral side of the fore-gut soon fuses with the ectoderm below it in a limited region to form the *oral plate* (Fig. 171); elsewhere between the ectoderm and endoderm of this vicinity, there are scattered mesoderm cells, i.e., mesenchyme.

DIFFERENTIATION OF THE EMBRYONIC MESODERM IN THE AREA PELLUCIDA

THE SOMITES AND LATERAL PLATES

The lateral sheets of mesoderm of the area pellucida now become thickened along either side of the head process and primitive streak. The ridges thus formed are known as the *vertebral* or *segmental plates*, while the remaining lateral portions of the sheets are called the *lateral plates*. Just in front of the anterior end of the primitive streak a transverse fissure now appears in each of the vertebral plates. The region of the plates immediately anterior to these fissures then constitutes the *first pair of somites*; they remain continuous anteriorly with the mesoderm

of the head region (Fig. 172). Slightly behind the first pair of fissures a second pair develops, and the part of the vertebral plates between the first and second pairs of fissures is the second pair of somites. The exact number of somites, and correlated development, varies consider-

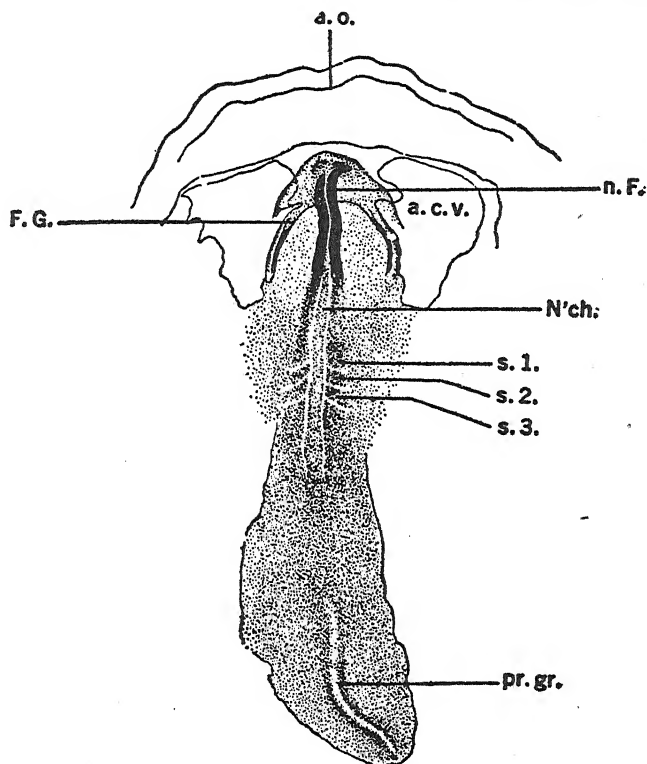


Fig. 172. — Chick embryo with three pairs of somites (about 23 hours). Dorsal view. From Lillie (*Development of the Chick*).

a.c.v. Amnio-cardiac vesicle. a.o. Inner margin of area opaca. F.G. Fore-gut. N'ch. Notochord. n.F. Neural fold. pr.gr. Primitive groove. s₁, s₂, s₃. First, second, and third somites.

ably, especially in the early stages, due to the breed of hen, the condition of the egg at laying, the precise temperature and other factors. At the end of 24 hours, however, there are usually from three to six of them — often about four — lying anterior to the primitive streak and hence upon either side of the head process, i.e., the rudiment of the notochord. The first four pairs of these somites later disappear, being included in the posterior part of the head.

The Nephrotome. — A narrow strip of each lateral plate immediately adjacent to the somites serves, as it were, to unite them to the main part of the plate. It is known as *the nephrotome*, and later gives rise to the excretory organs.

THE COELOM

Within the lateral sheets, which for a time remain connected with the somites by means of the nephrotomes, horizontal splits now develop. They occur first in the anterior portions and gradually spread elsewhere. Of the two sheets thus formed, the one next to the ectoderm is the *somatic* or *parietal mesoderm* (*somatopleure*), and that next to the endoderm the *splanchnic* or *visceral mesoderm* (*splanchnopleure*). The space between them is the *coelom* (Fig. 170).

THE RUDIMENT OF THE PERICARDIAL CAVITY

In the region of the head fold, the coelomic spaces on each side push toward each other. By so doing, they finally work their way in between the ectoderm and endoderm just at the bend where these two layers pass up from the depressed area under the fold on to its ventral surface. At the end of 24 hours, the walls of the opposite spaces have met each other and fused, so that the spaces themselves are separated only by a thin layer of mesoderm. This process tends to separate the ectoderm and the endoderm by pushing the latter further back, and thus increasing the length of the fore-gut. These in-pushing portions of the coelom are the *amnio-cardiac vesicles*, and they represent the rudiment of the *pericardial cavity* (Figs. 172, 183).

THE NERVOUS SYSTEM

Among the most conspicuous features of the early embryo is the rudiment of the central nervous system. This system first appears in the following manner:

THE MEDULLARY OR NEURAL PLATE

Beginning almost at the anterior limit of the head fold the ectoderm above and along each side of the head process is thickened somewhat; this thickening is the *medullary plate*. Posteriorly, the lateral portions of the plate extend also along each side of the primitive streak (groove). while the central portion merges with the ectoderm of the groove.

THE MEDULLARY GROOVE AND MEDULLARY FOLDS

Presently a depression appears running down the middle of the medullary plate above the head process, and on each side of this depression, the lateral portions of the plate rise up as two parallel ridges. The depression is, of course, the *medullary* or *neural groove*, while the ridges are the *medullary* or *neural folds* (Fig. 172). Approximately at the anterior end of the plate, the ends of the folds meet one another. However, because of the fact that they are already quite close together, this meeting does not form an extensive transverse ridge as in the Frog. Posteriorly, the folds do not at first reach quite to the region of the first somite, but before the end of the day they have extended backward to about the anterior end of the shortened primitive streak.

THE NEURAL TUBE

The parallel medullary folds now bend toward one another until their crests meet and fuse a little distance posterior to the anterior limit of the head fold, in the region of the future mid-brain. As in the case of the Frog, a continuation of this fusion results in the formation of a thick-walled tube, whose roof, sides, and floor are derived from the inner walls of the medullary folds and from the groove; it is the *neural tube* and its cavity of course is the *neural canal*. As in the Frog, also, there occurs shortly after the fusion of the folds, a separation between their inner (neural) and outer walls, the latter reconstituting above the tube a continuous layer of ectoderm.

These processes continue both anteriorly and posteriorly until the tube is entirely closed in. During the closure, however, the usual anterior and posterior openings into the neural canal persist. The former is the *neuropore*, corresponding to the structure of that name in the forms previously studied; this opening is closed during the first day. It should also be noted that because of the protrusion of the folds in this region, they extend forward slightly beyond the anterior limit of the fore-gut (Fig. 172). Later, as growth proceeds, this region is actually carried over the anterior end of the embryo on to the ventral side (see below under flexures). Posteriorly fusion takes place more rapidly, keeping pace with the extension of the medullary folds. Because of the greater distance to be traversed, however, the process in this direction is not completed until some time later. The completion at this end is marked by the disappearance of the primitive streak (Fig. 173).

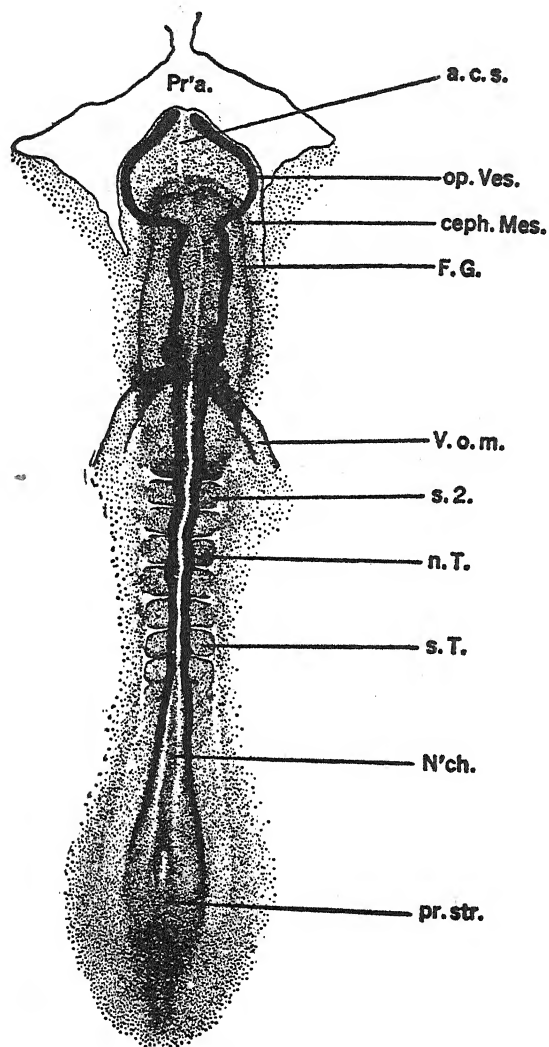


Fig. 173. — Chick embryo with seven pairs of somites (about 26–27 hours). Dorsal view. From Lillie (*Development of the Chick*).

a.c.s. Anterior cerebral suture; i.e., line of fusion of neural folds here. *ceph.Mes.* Cephalic mesoderm. *F.G.* Fore-gut. *N'ch.* Notochord. *n.T.* Neural tube. *op.Ves.* Optic vesicle. *Pr'a.* Proamnion. *pr.str.* Primitive streak. *s.2,s.7.* Second and seventh somites. *V.o.m.* Omphalomesenteric (vitelline) vein.

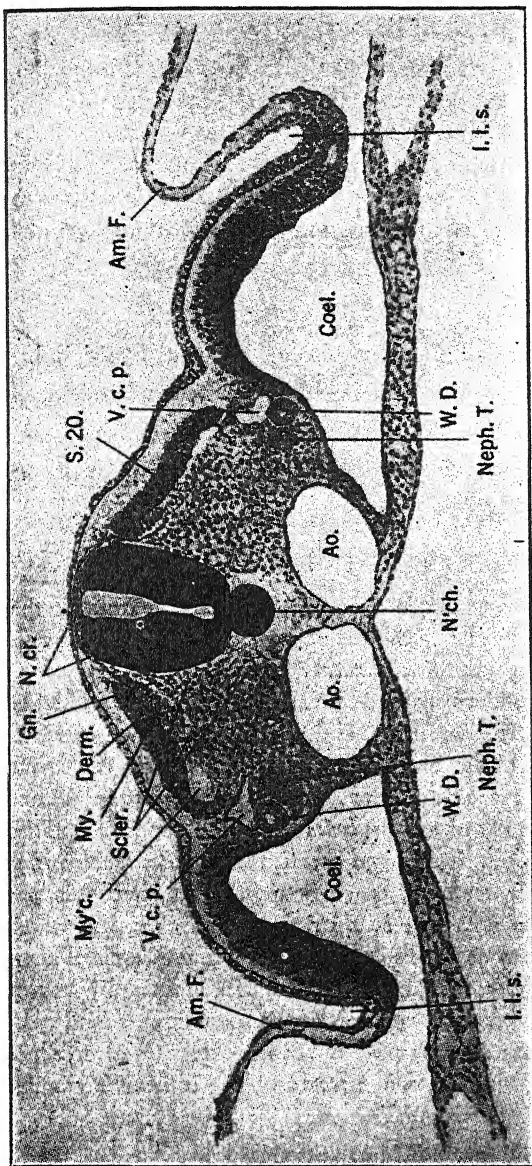


Fig. 174. — Transverse section through the twentieth somite of a 29 s embryo. From Lillie (*Development of the Chick*).
Am.F. Amniotic fold. *Ao.* Aorta. *Coel.* Coelom. *Derm.* Dermatome. *Gn.* Ganglion. *l.l.s.* Lateral limiting sulcus (see page 362). *My.* Myotome. *My'c.* Myocoel. *N'ch.* Notochord. *N'cr.* Neural crest. *Neph.T.* Nephrogenous tissue. *S.20.* Twentieth somite. *Scler.* Sclerotome. *V.c.p.* Posterior cardinal vein. *W.D.* Wolffian duct.

THE NEURAL CRESTS

At the same time that fusion of the folds is occurring, cells are proliferated between the outer and inner layers of each fold, just in the region of its crest. Thus, as fusion takes place, these cells form a band along either side of the dorsal part of the neural tube between it and the surface ectoderm. These bands are the *neural crests*, which at this time are united with one another across the dorsal surface of the tube (Fig. 174).

THE OPTIC VESICLES

Anterior to the first point of fusion, the neural tube is broadened somewhat. This is the region of the future *optic vesicles*.

SUMMARY OF THE CONDITION AT THE END OF THE FIRST DAY OF INCUBATION⁴

I. THE MESODERMAL STRUCTURES

About four pairs of *somites* are present, lying in front of the primitive knot and connected with the *mesoderm* of the respective *lateral plates* by the longitudinal *nephrotomal* bands.

The lateral mesoderm extends throughout the area pellucida except in the region of the *proamnion*, and together with the endoderm is being differentiated in the area opaca. In the latter area, the formation of this layer has progressed anteriorly until a pair of wing-like extensions are level with the tip of the head fold. Also in the area pellucida this mesoderm has been split into two sheets, the *somatopleure* and *splanchnopleure*, with the *coelomic* space between them, and this process is spreading into the area opaca. Beneath the fore-gut, the walls of the *amnio-cardiac* portions of the coelom have just met each other, and the rudiment of the *pericardial cavity* is thus indicated in this region.

In connection with the formation of the mesoderm, *blood vessels* and *corpuscles* have started to appear in the area opaca and area pellucida, transforming both into the *area vasculosa*. The latter is beginning to be bounded by the *sinus terminalis*.

⁴ Degree of development, including somite number, as noted, varies considerably, especially through 48 hours of incubation, and the hour or stage conditions designated in this text do not exactly agree with the carefully obtained results of Hamburger and Hamilton, '51. However, they are believed to correspond well with those indicated on the slides sold by most of the Biological Supply companies.

Outside the area vasculosa is an area consisting only of partially differentiated germ wall, the zone of junction, and the zone of overgrowth, the *area vitellina*.

II. THE HEAD FOLD AND THE FORE-GUT

The *head fold* has formed and in the process has given rise to the anterior or pharyngeal portion of the *fore-gut*.

III. THE RUDIMENTS OF THE NERVOUS SYSTEM

The *medullary folds* have appeared in the region in front of the primitive knot and have fused for a short space at their anterior ends. The *neural crests* have begun to appear, and the rudiments of the *optic vesicles* are also indicated.

T

HE CHICK: DEVELOPMENT DURING THE SECOND DAY OF INCUBATION

GENERAL APPEARANCE

THE embryos of the higher vertebrates, including Reptiles, Birds and Mammals, all develop in a more or less confined space, i.e., either within an egg shell or within the uterus. Also, in the early embryonic life, almost the anterior half of the organism in these forms is occupied by the brain which is growing very rapidly. Not only is this true, but the dorsal part of the mid-brain is growing with disproportionate rapidity, and this, combined with the confining space, causes a very marked bending of the entire anterior region. This bending presently leads also to a turning of the head end (torsion), and finally of the whole embryo, upon its side, as described below. Thus though the bending and turning are basically due to changes in the brain, and will be described in terms of that structure, it is convenient to do it under the heading of general external features.

FLEXURES AND TORSION

The Cranial Flexure. — The first bend, and one previously noted in connection with the brain of the Frog, is the *cranial flexure*. In the latter animal it was the only marked flexure of the brain, and had nothing to do with development in a confined space. Indeed the curve of this region of the brain was rather in part the remains of a portion of the original curvature of the egg. In the Chick and other higher animals the cranial flexure does not have this origin, but it does involve exactly the same regions of the brain, and the front of the embryo; i.e. it involves the fore-brain region which is bent down anterior to the notochord. This flexure begins at about thirty hours, and by the end of the day the bending is so great that the morphologically dorsal side of the mid-brain is actually the most anterior part of the embryo. The morphologically anterior side of the fore-brain, on the other hand, faces posteriorly so that this part of the embryo almost touches the heart (Figs. 175, 176). Finally, it should be noted that, as in the Frog, this flexure, in so

far as it concerns the brain, is permanent, and is the only one of those indicated at this time which is so.

The Cervical Flexure.—By the end of the day another broad curvature is evident, extending through the region of the hind-brain and back into the trunk. This is the *cervical flexure*, and has no counterpart in the Amphibian.

The Lateral Rotation or Torsion.—Finally as a result of both these flexures the front of the embryo would be thrust deep into the yolk were it not for a lateral twist which begins at the anterior end. By 48 hours it has progressed posteriorly about as far as the back end of the cervical flexure, i.e., approximately to the thirteenth somite. It is called the *lateral rotation* or *torsion*, and eventually results in turning the entire embryo over so that it lies upon its left side (Fig. 176).¹ It should be clearly understood in this connection that the terms dorsal, ventral and lateral in the present and following descriptions are used in their morphological sense. Thus dorsal will always refer to the side of the embryo upon which the nerve cord and notochord occur, and ventral will refer to the opposite side regardless of how the embryo lies.

LIMB BUDS

No limb buds are ordinarily visible at 48 hours. Nevertheless, if tissue from the locations where they would later appear is transplanted to

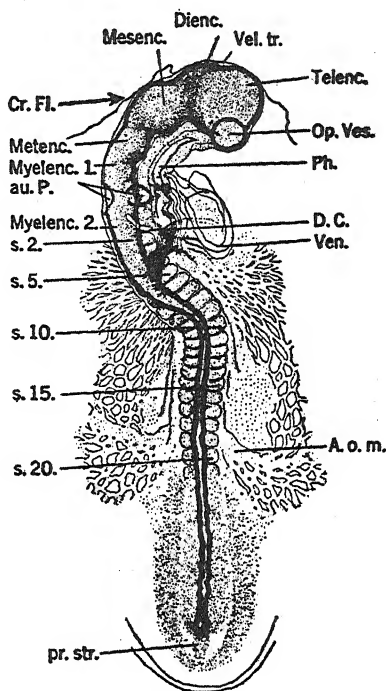


Fig. 175. — Chick embryo with twenty pairs of somites (about 43 hours). Dorsal view. From Lillie (*Development of the Chick*).

A.o.m. Vitelline artery. *au.P.* Auditory pit. *Cr.Fl.* Cranial flexure. *D.C.* Ductus Cuvieri. *Dienc.* Diencephalon. *Mesenc.* Mesencephalon. *Metenc.* Metencephalon. *Myelenc. 1* and *2.* Anterior and posterior divisions of the myelencephalon. *Op.Ves.* Optic Vesicle. *Ph.* Pharynx. *pr.str.* Primitive streak. *s.2.s.5.,* etc. Second, fifth, etc., somites. *Telenc.* Telencephalon. *Vel.tr.* Velum transversum. *Ven.* Ventricle.

¹ Occasional embryos are found lying upon the right side. Apparently this does not prevent subsequent normal development.

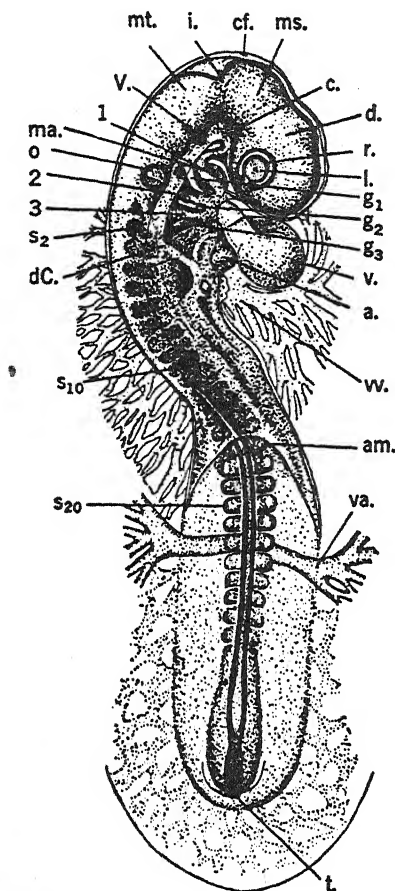


Fig. 176. — Chick embryo with twenty-seven pairs of somites (about 48 hours). From Kellicott (*Chordate Development*). After Lillie.

a. Auricle. am. Posterior margin of amniotic folds. c. Carotid loop. cf. Cranial flexure (cervical flexure also shown, see p. 333). d. Diencephalon. dC. Ductus Cuvieri. g_1 , g_2 , g_3 . Visceral clefts. i. Isthmus. l. Lens. ma. Mandibular arch. ms. Mesencephalon. mt. Metencephalon. o. Otocyst; to right of otocyst is ganglion of VII and VIII cranial nerves. r. Retinal layer. s_2 , s_{10} , s_{20} . Second, tenth, and twentieth somites. t. Tail-bud. v. Ventricle. va. Vitelline artery. vv. Vitelline vein. 1, 2, 3. First, second, third aortic arches. V. Ganglion V cranial nerve.

other locations it will produce there either a wing or a hind limb depending upon its source. Furthermore, the dorso-ventral and antero-posterior axes of these transplanted tissues will not have been altered, i.e., such potential limb tissue (anlage) transplanted in an inverted position will produce an inverted limb. Thus it appears that the destiny of this tissue has already been rather completely determined. It will not only form a limb, but a limb of a particular type which retains all its original axes (Hamburger, '38).

THE SOMITES

When last mentioned, the somites were described as masses of mesoderm connected with the lateral plates by means of the nephrotomes. During the second 24 hours the connection between nephrotome and somite is obliterated throughout the greater part of the embryo; the number of pairs of the latter increases to 27, and beginning at the anterior end the development of each of the somites proceeds in the following manner:

THE MYOTOMES AND THE CUTIS PLATES

Each somite is at first composed of an outer layer of comparatively dense cells surrounding an inner mass of mesenchyme, the latter

comparable to a *myocoel*, so far as one exists (Fig. 170, B). Presently, however, the denser layer of cells on the side of the somite next to the nerve cord and notochord largely disappears, leaving the latter structures in direct contact with the mesenchymatous mass indicated above. At the same time the dense layer upon the dorsal and outer side of the somite becomes thicker. The dorsal portion of this outer layer is the rudiment of the *myotome*, while the more lateral and ventral portion is the *cutis plate* or *dermatome*. Before the second day has passed, the dorsal or myotomal portion of the above plate of cells begins to turn sharply upon itself and grow downward between the mesenchyme and the cutis plate. Thus a double layer of cells begins to be formed consisting of the cutis plate on the outside and the myotomal plate on the inside (Fig. 174).

THE SCLEROTOME

The mesenchyme which now begins gradually to surround the notochord and the ventro-lateral region of the nerve cord is the rudiment of the *scl otome*.

THE ALIMENTARY TRACT

THE FORE-GUT

The Stomodaeum. — During the first day it was noted that the antero-ventral end of the fore-gut came in contact with the ectoderm at a point on the ventral side of the head fold to form the oral plate. Now, as the result of the downward flexure of the head and also of the pushing forward of the mandibular arches (see below), the central region of the plate becomes relatively depressed to form a pit lined by ectoderm. It is the beginning of the *stomodaeum*, and by a continuation of the above process it presently acquires a considerable depth.

Rathke's Pocket. — From the antero-dorsal wall of the stomodaeum a small diverticulum now appears growing anteriorly along the morphologically ventral side of the posterior portion of the fore-brain which has been bent down in front of it. It is called *Rathke's pocket*, and is destined to become the anterior part of the hypophysis or pituitary. (See the footnote on this under the Frog.)

The Visceral Pouches and Arches.

The Pouches. — In the anterior or pharyngeal portion of the fore-gut, a series of vertical folds of the endodermal wall begin to push out toward the ectoderm on each side of the head. These are the *visceral*

pouches, and they develop in regular order, the most anterior pair appearing first. The first pair are known as the *first visceral* or *hyomandibular* pouches, and the remaining pairs, of which there are three, as the *second*, *third*, and *fourth visceral* ("branchial") pouches. They decrease in size posteriorly, the last pair being relatively small. The first pair of pouches, i.e., the hyomandibulars, fuse with the corresponding ectodermal invaginations (*visceral furrows*) only at their dorsal ends, while the second and third pairs fuse with their respective furrows throughout their lengths, except for a slight interruption in their upper halves. The point of fusion of the first pouch now becomes perforated as the first or *spiracular cleft*. The fusion of the fourth pair of pouches and furrows, and the perforation at the points of fusion of the second and third pairs to form actual *visceral clefts*, occurs later (Figs. 176 and 194).²

The Arches. — Anterior and posterior to each pouch the mesenchyme becomes thickened to form the *visceral arches*. The arch in front of the first or hyomandibular pouch is the *first visceral* or *mandibular* arch, and the one between it and the second pouch is the *second visceral* or *hyoid* arch. The remainder are simply the *third*, *fourth*, and *fifth visceral* ("branchial") arches, and they appear in the same order as the pouches; the fifth and last arch is hardly more than a transitory vestige. Presently, blood vessels and nerves pass into the arches, as will be indicated later.

It should be noted in passing, that though these pouches and arches correspond to the similarly developed structures in the Frog, in this case no gills ever appear in connection with any of them. The term *visceral* rather than *branchial* is therefore more aptly applied to them all.

The Thyroid. — This begins to develop near the end of the second day as a small thickening in the middle of the floor of the pharynx, between the ventral ends of the second pair of visceral arches. Before the end of the day it has become slightly evaginated so as to form a shallow depression in the pharyngeal floor (Fig. 184).

² According to a recent investigator (Dudley, '42) there are actually six visceral pouches in the Chick embryo, but the last two are very early merged with the fourth to form what this author calls the "fourth visceral complex," the "sixth pouch" component later forming the post-branchial body (see below). As will be noted later, others have regarded the primordial lung outgrowths as fifth visceral pouches. It appears to the present writer that these are all somewhat forced attempts to make the situation in the Bird square more nearly with that in some of the lower Chordates. Whether either the lung outgrowths or the rudimentary structures referred to by Dudley really represent any visceral pouches or not, is, the writer believes, still open to considerable question.

The Respiratory System.—Late the second day a longitudinal groove, with a pair of slight posterior expansions, appears in the floor of the pharynx caudal to the visceral pouches. It is the beginning of the larynx, the trachea, and the lungs, and thus represents the start of the entire respiratory system. In this connection it may be recalled that according to one view the lung primordia of the Frog are to be homologized with a hypothetical seventh pair of gill pouches. It is therefore of interest to find that in this case the above expansions which later develop into the lung primordia of the Chick are similarly homologized by some with a fifth pair of visceral pouches. (See, however, preceding footnote.)

The Liver.—Just at the posterior limit of the fore-gut behind the pharyngeal region, there appear at this time two slight antero-ventrally directed evaginations of the endoderm whose development is said to depend on contact with the veins (cardiac primordia) in this region (Willier, and Rawles, '31). The diverticula are not of course suspended in space, but pushed forward into the mass of splanchnic mesoderm (*ventral mesentery*) which unites the gut and the ductus venosus in this vicinity. One of the diverticula is a little in advance of the other both in position and in time of appearance. It presently pushes forward so as to lie just dorsal to the point of union of the vitelline veins (see below), while the other, at this period, is barely distinguishable. These two diverticula represent the rudiments of the *liver*.

THE MID-GUT

There is little indication of any real mid-gut during the second day, but rather merely a wide enteric space overlying the yolk. The beginning of folds along the sides of the embryo continuous with the lateral margins of the head fold suggests, however, the manner in which this portion of the gut will be formed.

THE HIND-GUT

The Posterior Intestinal Portal and Anal Plate.—At the close of the second day the hind-gut begins to develop in connection with a tail fold very similar to the head fold. There is thus formed a posteriorly directed cavity lined by endoderm, and lying beneath the remains of the primitive streak. It is the *hind-gut*, and opens anteriorly into the wide enteric space overlying the yolk (rudiment of the mid-gut). As in the case of the fore-gut, the region of this opening is termed an intestinal portal—in this instance, the *posterior intestinal portal*. There is fi-

nally one further resemblance between fore- and hind-guts in that at the end of the latter the endoderm comes in contact with the ectoderm and fuses with it. This point of fusion is at the posterior end of the primitive streak, and marks the location of the future anus. It is termed the *anal plate* or *cloacal membrane*. Besides these points of resemblance, there are now to be noticed certain important differences as follows (Fig. 177):

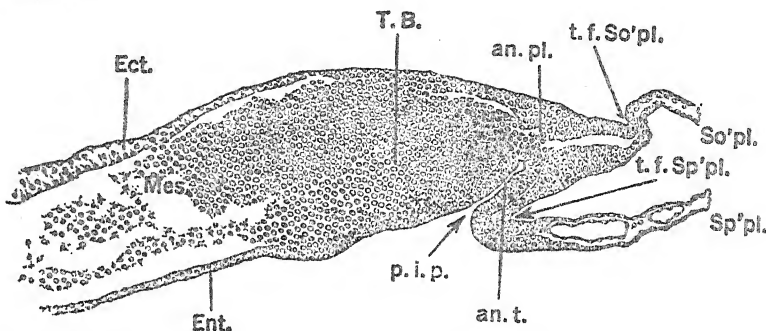


Fig. 177. — Median longitudinal section through the hind end of an embryo of about 21 s. From Lillie (*Development of the Chick*).

an.pl. Anal plate. *an.t.* Anal tube (hind-gut). *Ect.* Ectoderm. *Ent.* Endoderm. *Mes.* Mesoderm. *p.i.p.* Posterior intestinal portal. *T.B.* Tail-bud. *t.f.So'pl.* Tail fold in the somatopleure and ectoderm. *t.f.Sp'pl.* Tail fold in the splanchnopleure and endoderm.

The Ventral Mesentery. — It has been stated that the hind-gut is formed in connection with a tail fold, just as the fore-gut is formed in connection with the head fold, and in a general way this is true. In the case of the tail fold, however, there is this difference. The endoderm is folded in to form the hind-gut and the intestinal portal, but in this case the ectoderm follows this infolding much more slowly than it did in the case of the head fold. Thus it happens that the hind-gut arises before there is any very marked indication of a tail fold on the surface of the blastoderm. For this reason the anal plate, unlike the oral plate, remains dorsal for some time, and is only gradually carried around onto the ventral surface (Fig. 177).

Furthermore, this lagging behind of the ectodermal portion of the fold necessarily means that there is a gap between the two cell layers; this gap in the case of the tail fold is filled by mesoderm. Presently lateral extensions of the embryonic coelom press back into this region upon either side, but for a time they do not meet one another. Thus there is left a median mesodermal mass extending from the ventral side

of the hind-gut backward and upward to the underside of the lagging ectoderm. That portion in contact with the gut may be referred to as splanchnic, and that in contact with the ectoderm as somatic. The two portions are continuous, however, and together are known as the *ventral mesentery* of the hind-gut.

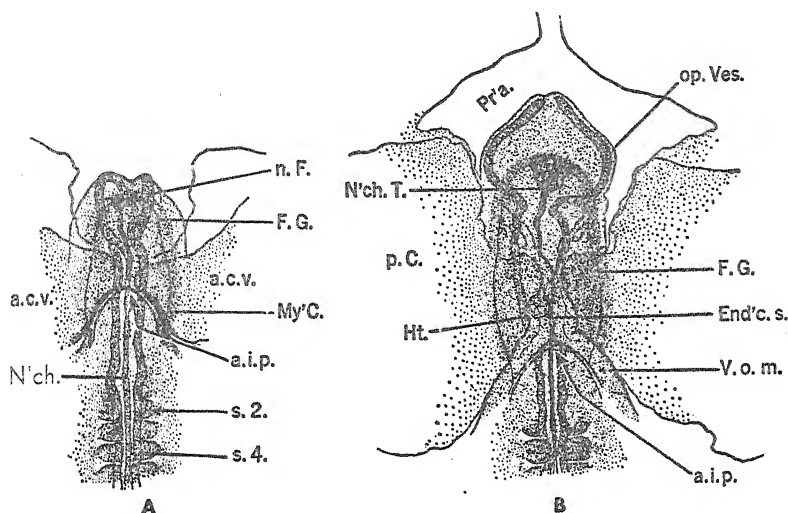


Fig. 178. — Ventral views of the head ends of Chick embryos. From Lillie (*Development of the Chick*). A. Embryo with five pairs of somites (about 23 hours). B. Embryo with seven pairs of somites (about 25 hours).

a.c.v. Amnio-cardiac vesicle. a.i.p. Anterior intestinal portal. End'c.s. Endocardial septum. F.G. Fore-gut. Ht. Heart. My'C. Myocardium. N'ch. Notochord. N'ch.T. Anterior tip of notochord. n.F. Neural fold. op.Ves. Optic vesicle. p.C. Pericardial cavity (amnio-cardiac vesicles). Pra. Proamnion. s₂.s₄. Second and fourth mesodermal somites. V.o.m. Omphalomesenteric vein.

THE CIRCULATORY SYSTEM

THE HEART

The Origin and the Formation of the Endothelial Lining. — While blood vessels and corpuscles have been developing from the germ wall in the area opaca, vessels have also begun to form in the area pellucida. These latter vessels, which are in direct continuity with those already formed, also arise from blood islands, though these islands are slightly different from those of the area opaca. They are merely aggregations of cells, apparently detached from the splanchnic mesoderm, and the vessels into which they develop are temporarily entirely devoid

of corpuscles. Erythrocytes, however, are soon supplied from the area opaca, and also by cells budded from the posterior ends of the dorsal aortae (Danchakoff, '07). Thus from the cell aggregates, as indicated, rudiments of two large vessels (the *omphalomesenteric* or *vitelline veins*)

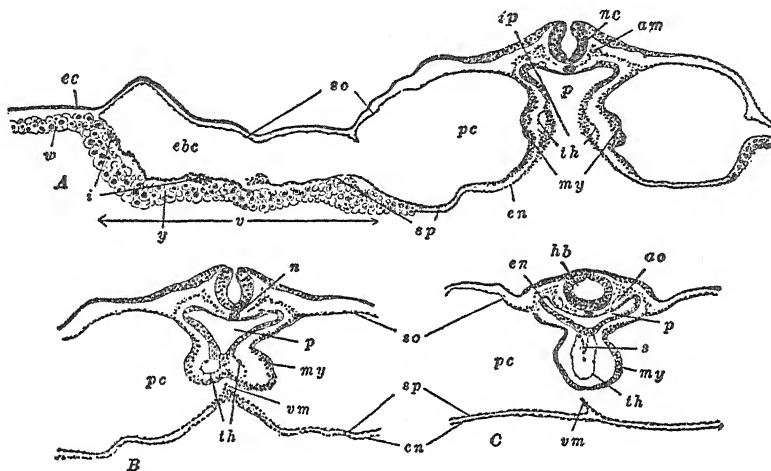


Fig. 179. — Sections through Chick embryos showing particularly the formation of the heart, pericardial cavity, and pharynx. From Kellicott (*Chordate Development*). After Lillie. *A*. Just posterior to the anterior intestinal portal of a Chick with seven pairs of somites (about 25 hours). *B*. Section just anterior to *A*. *C*. Through the heart of an embryo with ten pairs of somites (about 29 hours).

am. Axial mesodermal thickening. ao. Lateral dorsal aorta. ebc. Exocoelom. ec. Ectoderm. en. Endoderm. hb. Hind-brain. i. Blood islands. ip. Anterior intestinal portal. my. Myocardium (muscular layer of heart). n. Notochord. nc. Nerve cord. p. Pharynx. pc. Pericardial cavity (amnio-cardiac vesicles). s. Endothelial septum. so. Somatic mesoderm. sp. Splanchnic mesoderm. th. Cardiac endothelium. v. Area vasculosa. vm. Ventral mesocardium. w. Germ wall. y. yolk-sac endoderm.

soon appear in the area pellucida (Fig. 178): Each rudiment rests upon one of the ventro-lateral walls of the fore-gut, between it and the median-lateral wall of the respective amnio-cardiac vesicle from which it has arisen (Fig. 179, A).³ The anterior portions of these rudiments then form the endothelial lining of the heart in the following manner:

It is to be recalled that the amnio-cardiac vesicles have already become fused beneath the fore-gut, just in front of the endodermal wall

³ The evidence of this figure would seem to indicate that the vessels are derived from the walls of the gut rather than from those of the vesicles, and some authorities hold this to be the case. In view, however, of the origin of the other blood vessels of this area from the mesoderm, it seems more likely that the latter derivation is the true one.

which marks its posterior limit (Fig. 178, *A*). The fusion now progresses posteriorly, as it does so pushing back and closing in the ventro-lateral gut walls against which the veins indicated in the preceding paragraph are resting. Thus as these walls come together the anterior ends of the above mentioned vessels are likewise brought together side by side beneath the newly formed gut, and as this occurs they fuse with one another to form a single vessel with a median partition. This partition soon disappears, and the single median tube which remains is the *endothelial lining of the rudimentary heart* (Figs. 178, *B* and 179, *B*, *C*).

The Myocardium of the Heart. — The median walls of the amnio-cardiac vesicles which now lie against each side of the endothelial tube presently press in above and below it, and fuse with each other. Thus the tube is completely surrounded by mesoderm which forms the *myocardium* or muscular element of the heart, and its covering the visceral pericardium.

The Mesocardia. — The above fusion leaves the endothelial tube and its myocardium suspended from the mesodermal covering of the ventral wall of the fore-gut, or pharynx, by a double layered sheet of mesoderm (ventral mesentery) here termed the *dorsal mesocardium*. Ventrally also a similar sheet attaches the tube to the underlying splanchnic mesoderm. The latter quickly disappears, and the former does so later, except at the anterior and posterior ends of the heart (Fig. 179, *C*).

The Pericardial Cavity and Parietal Pericardium. — With the fusion and disappearance of the median walls of the amnio-cardiac vesicles, it is clear that their cavities have become a single space which surrounds the heart. This space is the *pericardial cavity*, and its walls constitute the rudiments of the greater part of the parietal *pericardium*. Postero-laterally, however, the pericardium is still incomplete, and hence the above cavity continues to communicate in this direction with the general coelom.

The Rudiments of the Atria, Ventricles, Bulbus and Truncus Arteriosus. — In connection with the description of the development of the Frog heart it was noted that the development of all Vertebrate hearts is essentially similar. This similarity has already become apparent as between the Frog and Chick in that the hearts of both start with the fusion of two vessels to form a tube. Further similarities will now reveal themselves in the transformations of this tube in the Chick to form the adult organ.

As in the Frog, the straight tube first increases in length, and, its ends being fixed, its middle bows laterally to the right (Figs. 180 and 181). The broad apex of the bow is then drawn ventrally, and usually slightly posteriorly, while the whole tube is at the same time thrown into a loop. (These terms of direction it should here be recalled are being used in the morphological sense regardless of the rotation of the embryo onto its side.) Again as in the Frog, the loop which has been produced in the originally straight tube lies to the right of the median line. This means that the posterior limb of the loop extends ventrally,

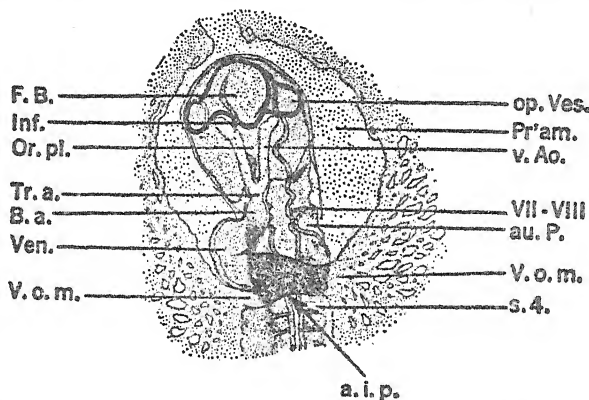


Fig. 180. — Ventral view of the anterior end of a Chick embryo with sixteen pairs of somites (about 38 hours). From Lillie (*Development of the Chick*).

a.i.p. Anterior intestinal portal. *au.P.* Auditory pit. *B.a.* Bulbus arteriosus. *F.B.* Fore-brain. *Inf.* Infundibulum. *op.Ves.* Optic vesicle. *Or.pl.* Oral plate. *Pr'am.* Proamnion. *s.4.* Fourth somite. *Tr.a.* Truncus arteriosus. *v.Ao.* Ventral aorta. *Ven.* Ventricle. *V.o.m.* Omphalomesenteric (vitelline) vein. *VII-VIII.* Acustico-facialis ganglion.

and as suggested, usually slightly posteriorly. The middle part then curves laterally toward the right, where it passes into the ascending limb which extends dorsally, anteriorly and medially back into the median plane (Figs. 108, 176). It now remains to indicate the parts of the future heart which the various regions of this loop are destined to form. Beginning at the posterior end the region where the posterior limb starts to descend will comprise the *atria*. The apex of the loop and a small portion of the descending and ascending limbs will become the *ventricles*. The larger part of the anterior ascending limb will become the *bulbus* and *truncus arteriosus*.

As regards the functioning of the Chick heart, the first indications of it have been found to occur about the twenty-ninth hour of incubation, and as in the Frog, long before any innervation. The contractions begin along the right side of the heart tube in the future ventricular region, and then spread to the left. As the atrial region forms behind the ventricular, the contractions also extend to it, and finally to the sinus venosus. As in the case of the Frog, experimental transections of the heart tube show that the inherent rate of contraction increases as one passes posteriorly. Also the most posterior region at any given stage acts as the pacemaker, while the older anterior regions gradually lose the power of automatic contraction. Thus the rate for the whole heart is slowly stepped up and is finally set by the sinus, which is ultimately incorporated into the right atrium (Patten and Kramer, '33, Barry, '42). Later on following innervation the rate of beat is of course partially under nervous control.

THE BLOOD VESSELS OF THE EMBRYO

The Arteries.

The Dorsal Aortae and Their Branches. Along each side of the embryo, just at the inner margin of the pellucid area, two vessels now develop. These are the *dorsal aortae* (Fig. 181, A). Anteriorly each is continued into a vessel differentiated in the mesenchyme on either side of the head. Posteriorly they give off branches between the somites (*segmental arteries*), and finally leave the sides of the embryo at about the level of the seventeenth somite to pass out into the general vascular network as the *vitelline arteries*. Near the end of the second day the two dorsal aortae fuse with one another in the region above the heart, forming for a short distance a single dorsal vessel.

Development of the Aortic Arches.—The truncus arteriosus at first runs anteriorly a short distance, this short relatively horizontal extension being called a ventral aorta. It is, however, merely a continuation of the truncus, and is presently so incorporated with it that there is no distinction. At its anterior end this short extension of the truncus divides into two vessels which extend still further forward in the pharyngeal floor. They also are frequently called ventral aortae. As will presently appear, however, their proximal portions really constitute the proximal parts of the first pair of aortic arches (Figs. 180, 176). Somewhat anterior to the oral plate each of these vessels bends sharply upward to join the respective dorsal aorta, this bend being termed the

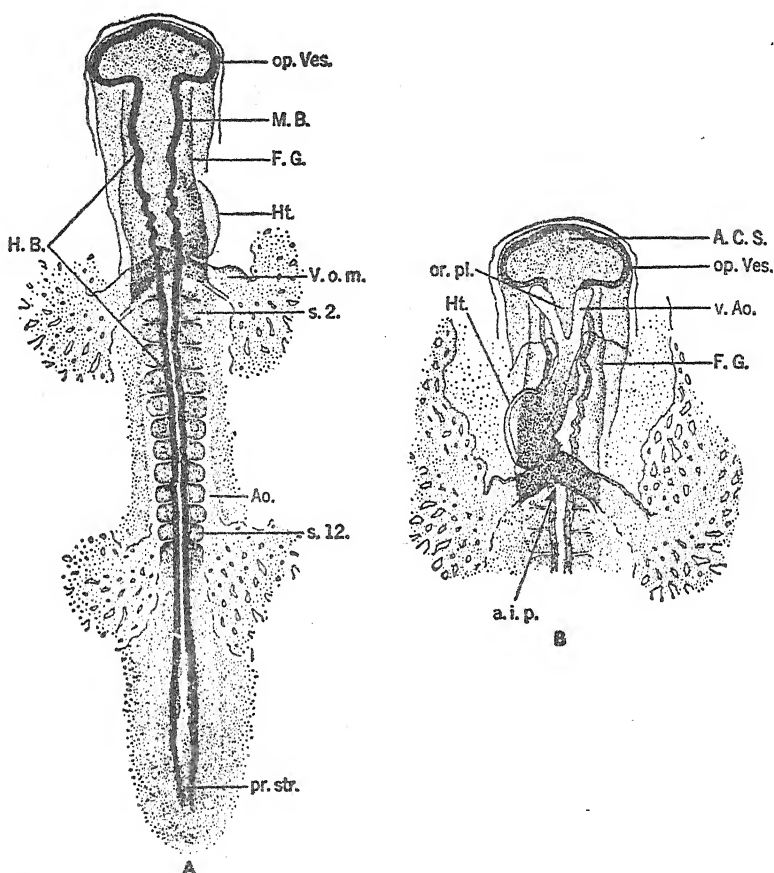


Fig. 181. — Chick embryo with 12 pairs of somites (about 33 hours). From Lillie (*Development of the Chick*). A. Dorsal view of entire embryo. B. Ventral view of anterior end.

A.C.S. Anterior cerebral suture. a.i.p. Anterior intestinal portal. Ao. Dorsal aorta. F.G. Fore-gut. H.B. Hind-brain. Ht. Heart. M.B. Mid-brain. op.Ves. Optic vesicle. or.pl. Oral plate. pr.str. Primitive streak. s_2 s_{12} . Second and twelfth somites. v.Ao. Ventral aorta. V.o.m. Omphalomesenteric vein.

carotid loop. Meanwhile, as previously indicated, the visceral pouches and arches have been forming, and in the arches certain blood vessels have been developing on each side as follows:

In the first place the single or common ventral aorta has, as predicted, become incorporated into the truncus whose wide dorsal end now terminates directly beneath the visceral arches. While this has been

occurring each first or mandibular arch has pushed ventrad. As a result of this the proximal part of each of the separate ventral aortae comes to lie within about the ventral four-fifths of the respective mandibular arch. Thus, as suggested above, this part of each ventral aorta comes to form the proximal portion of each *first aortic arch*. The more distal fifth of each first aortic arch which will lie within the corresponding distal fifth of the mandibular arch, remains for the time being incomplete. The proximal four-fifths of this vessel is, however, still connected with the dorsal aorta by way of the remaining anterior tip of the respective ventral aorta and carotid loop as previously indicated (Fig. 176). The actual completion of the distal portion of the first aortic arch so that this artery lies entirely within the mandibular arch apparently does not occur until the third day, and will be described when that stage is reached. The development of the remaining aortic arches is more straightforward. The *second aortic arches* develop in the second visceral or hyoid arches, and the *third aortic arches* develop in the third visceral arches. These last pairs arise as buds from the dorsal aortae which grow almost directly ventrad through the arches to join the dorsal end of the truncus.

The Veins and the Lateral Mesocardia. — As has been indicated above, the endothelial portion of the heart is formed by the growing together of two large vessels (omphalomesenteric veins). It now remains to state that this union continues for a short distance posterior to the atrial rudiments. The most anterior part of this continuation is somewhat dilated and is known as the *sinus venosus*, while slightly further back it receives the name of *ductus venosus*. The most anterior portion of the sinus venosus is sometimes regarded as part of the heart proper, because later it is involved in the development of the right atrium. At this stage, however, it may best be considered as a part of the venous system.

During the second day there develops in the mesenchyme on each ventro-lateral side of the brain a vessel which runs posteriorly as far as the level of the heart. These are the *anterior cardinal veins*. Meantime there has occurred on each side of the embryo a fusion of the lateral body wall with the posterior part of the sinus venosus. Thus a pair of septa have been formed each of which passes somewhat diagonally laterally and dorsally from the posterior part of the sinus to the respective body wall. These are called the *lateral mesocardia*, and within each of them develops a rather large vein, the *ductus Cuvieri* (Figs. 176; 182, C). Each ductus Cuvieri connects ventrally with the sinus venosus and dor-

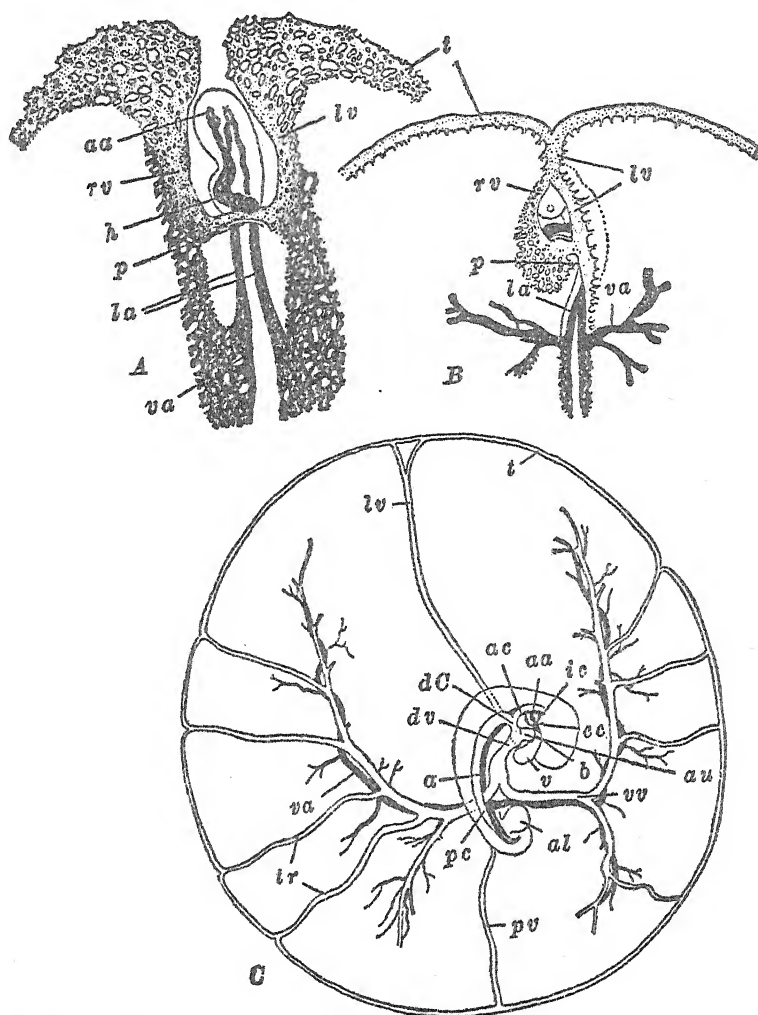


Fig. 182. — Diagrams of the circulation in the Chick embryo and area vasculosa. From Kellicott (*Chordate Development*). The vascular network of the area vasculosa is omitted for the most part. *A*. Anterior and central parts of the embryo and vascular area at about thirty-eight hours (sixteen pairs of somites). Viewed from beneath. After Popoff. *B*. Median and anterior parts of vascular area and embryo at about seventy-two hours. (twenty-seven pairs of somites; the number is usually nearer to 36 at this age). Viewed from beneath. After Popoff. *C*. The main vascular trunks of the fourth day. After Lillie (modified).

a. Dorsal aorta. *aa*. Aortic arches (first and second in *A*, second, third, and fourth in *C*). *ac*. Anterior cardinal vein. *al*. Allantois. *au*. Atrium. *b*. Bulbus arteriosus. *dc*. Ductus Cuvieri. *dv*. Ductus venosus. *ec*. External carotid artery. *h*. Heart. *ic*. Internal carotid artery. *la*. Lateral dorsal aorta. *lv*. Left anterior vitelline vein. *p*. Anterior intestinal portal. *pc*. Posterior cardinal vein. *pv*. Posterior vitelline vein. *rv*. Right anterior vitelline vein. *t*. Sinus terminalis. *tr*. Venous trunks of the area vasculosa. *v*. Ventricle. *va*. Vitelline artery. *vv*. Vitelline or omphalomesenteric vein (in this region really lateral vitelline vein).

sally with the posterior end of the respective anterior cardinal vein. From this point of union still another vein grows posteriorly along each side of the body. These veins are known as the *posterior cardinals* (Fig. 182, C).

THE EXTRA-EMBRYONIC BLOOD VESSELS

Extension of the Area Vasculosa and the Mesoderm.—By about the end of the second day the two anterior wings of the area vasculosa, and the extra-embryonic mesoderm and entoderm which accompany them, have bent toward one another and have fused in front of the proamnion. The area vasculosa, therefore, now entirely surrounds the latter region, and is itself completely encircled by the sinus terminalis, which has been referred to above (Fig. 182, A, B). Meanwhile, certain veins and arteries have extended from the embryo into the vascular area, as follows:

At the posterior end of the ductus venosus, the union of the vessels which form it terminates, and each passes outward into the area pellucida. At this point they are known as the vitelline or omphalomesenteric veins. Upon coming into this region each of the veins turns anteriorly and runs past the head around the inner boundaries of the approaching wings of the area vasculosa. Hence these extensions are known as the right and left *anterior vitelline veins*. First by a system of capillaries, but presently directly, each of these veins then becomes connected with the anterior extremities of the sinus terminalis. It thus happens that as the vascular wings meet one another, the sinus terminalis not only becomes complete, but the ends of the two anterior vitelline veins also meet and form one vessel (Fig. 182). At the proximal ends of these veins each gives rise during this period to a slight lateral outgrowth—the beginnings of the *lateral vitelline veins*.

The vitelline arteries, already referred to, extend out into the lateral portions of the area vasculosa some distance back of the vitelline veins, i.e., by the end of the day at about the twentieth somite.

THE CIRCULATION AS ESTABLISHED ON SECOND DAY

It will now be seen that with the establishment of the capillary network within the area vasculosa, and the formation of the arches connecting the ventral and dorsal aortae within the embryo, a complete system of circulation has been made possible. The further development of this system will be described as it occurs.

THE NERVOUS SYSTEM

THE MAIN DIVISIONS OF THE EARLY BRAIN

Early on the second day of incubation a slight constriction appears just back of the optic vesicles, marking the posterior boundary of the *fore-brain* or *prosencephalon*. Presently this is followed somewhat further back by another constriction which marks the posterior limit of

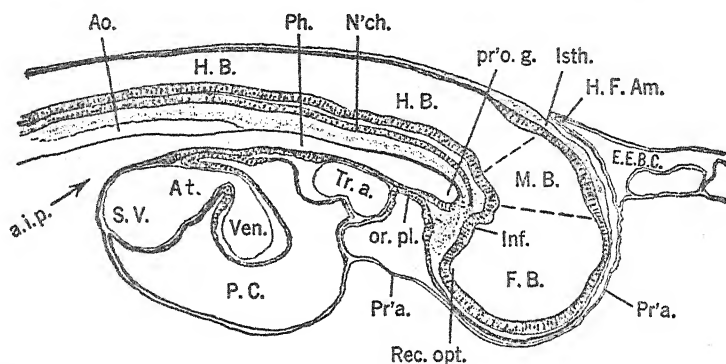


Fig. 183. — Median sagittal section through the head end of a Chick with 18 pairs of somites (about 40 hours). From Lillie (*Development of the Chick*).

a.i.p. Anterior intestinal portal. *Ao.* Dorsal aorta. *At.* Atrium. *E.E.B.C.* Exocoelom (extra-embryonic body cavity). *F.B.* Fore-brain. *H.B.* Hind-brain. *H.F.Am.* Head-fold of amnion. *Inf.* Infundibulum. *Isth.* Isthmus. *M.B.* Mid-brain. *N'ch.* Notochord. *or.pl.* Oral plate (oral membrane). *P.C.* Pericardial cavity. *Ph.* Pharynx. *Pr'a.* Proamion. *pr'o.g.* Preoral gut. *Rec.opt.* Optic recess. *S.V.* Sinus venosus. *Tr.a.* Truncus arteriosus. *Ven.* Ventricle.

the *mid-brain* or *mesencephalon*. The part posterior to this is the *hind-brain* or *rhombencephalon* which passes insensibly into the region of the spinal cord. The posterior limit of the hind-brain, however, may be fixed in a general way at this time by the position of the fourth somite (Figs. 181, 183). It should again be noted that the cranial and cervical flexures are especially concerned with the brain. As suggested, however, because that organ occupies so large a part of the anterior of the embryo at this stage these flexures affect the whole organism in this region and were therefore described under general appearances.

THE FORE-BRAIN OR PROSENCEPHALON

Its Extent. — On the posterior wall, i.e., on the floor of that part of the brain where the cranial flexure is most pronounced, at the end of the slightly bent notochord, is an invagination. It is directed antero-

ventrally into the cavity of the brain, and is called the *tuberculum posterius* (Fig. 184). On the opposite or anterior wall of the brain a little below the level of this evagination is the slight, but broad, constriction referred to above as marking the posterior boundary of the fore-brain. This boundary may now be more accurately defined as a plane passing from the tuberculum posterius on the posterior wall to the mid-point of the broad constriction on the anterior wall. This mid-point marks also the position of the future posterior commissure (see fourth day).

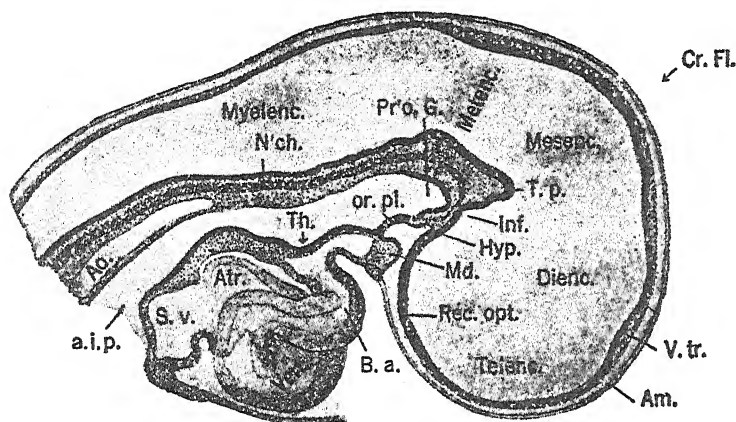


Fig. 184. — Optical sagittal section of the head of an embryo of 22-23 s. The heart is represented entire. From Lillie (*Development of the Chick*).

Atr. Atrium. B.a. Bulbus arteriosus. Cr.Fl. Cranial flexure. Dienc. Diencephalon. Hyp. Rathke's pocket, rudiment of anterior hypophysis. Inf. Infundibulum. Md. Mandibular arch. Metenc. Metencephalon. Myelenc. Myelencephalon. or.pl. Oral plate. Pr'o.G. Preoral gut. Th. First indication of thyroid. Rec.opt. Optic recess. Telenc. Telencephalon. T.p. Tuberculum posterius. V.tr. Velum transversum.

Parts of the Fore-brain.

The Infundibulum. — Just ventral to the tuberculum posterius, a small posteriorly directed evagination now appears lying slightly beneath the anterior end of the notochord. It is the beginning of the *infundibulum*, the future posterior part of the pituitary (Fig. 184).

The Region of the Optic Vesicles. — Ventral to the infundibulum, but still on the posterior wall, is a thickening, the rudiment of the future *optic chiasma* (not noticeable in Fig. 184), while immediately ventral to this thickening is a small evagination, the *optic recess*. From this recess the hollow optic vesicles have grown out on either side, and as they have grown their proximal parts have been constricted, as in the case of the

Frog, to form the *optic stalks*. Below the optic recess, the posterior wall begins to curve anteriorly onto the present ventral surface. This region is relatively thin and is known as the *lamina terminalis*. Within it the torus transversus is scarcely visible as yet.

The Cerebral Hemispheres. — Near the end of the second day the sides of the fore-brain just dorsal to the lamina terminalis begin to push out as the future *cerebral hemispheres*. Their cavities will be the *lateral ventricles* opening into the cavity of the fore-brain or *third ventricle*, through the *foramina of Monro*.

The Velum Transversum and Region of Epiphysis. — Beyond the region of the lamina terminalis on the antero-ventral side of the fore-brain, we come to a portion of the wall which is slightly depressed. It is known as the *velum transversum*. Further dorsal to this point on approximately the anterior surface may be found, also, the suggestion of an outpushing; it marks the general region from which the epiphysis (pineal gland) later (fourth day) arises. This brings us to the slight but broad constriction mentioned above as indicating the location of the future posterior commissure, and the limit of the fore-brain.

The Divisions of the Fore-brain. — As in the case of the Frog, it is customary to divide the fore-brain into two parts, which with the aid of the above landmarks may now be easily defined. That part of the fore-brain which lies ventro-anterior to a plane passing from the posterior wall just ventral to the optic recess to the anterior wall slightly anterior to the middle of the velum transversum is the *telencephalon*. The remaining portion of the fore-brain, whose posterior limit is defined above, is then the *diencephalon*. The cerebral hemispheres arise from the former.

THE MID-BRAIN OR MESENCEPHALON

The anterior boundary of the mesencephalon coincides with the posterior boundary of the diencephalon, marked by the broad constriction previously referred to. The posterior boundary may be defined as a transverse plane passing from the postero-ventral wall or floor just above and behind the tuberculum posterius, upward to about the middle of another rather broad constriction on the antero-dorsal wall (Fig. 184). The roof of the mid-brain, moreover, is growing so rapidly in connection with the cranial flexure, that it soon arches outward as the most anterior region of the embryo. Other parts of the mesencephalon have not appeared, and will, therefore, be described later as they arise.

THE HIND-BRAIN OR RHOMBENCEPHALON

Its Extent. — The hind-brain lies entirely dorsal to the notochord, and extends from the constriction marking the boundary of the mid-brain posteriorly into the spinal cord. Its posterior boundary, as stated above, can be defined only as that part opposite the fourth somite. As in the case of the mid-brain, the parts of the hind-brain are not yet discernible, and will be indicated when they appear.

The Divisions of the Hind-brain. — The divisions of the hind-brain are also difficult to define at this early stage. We may say, however, that the anterior division is relatively short, and is known as the *metencephalon*. The remainder of the brain constitutes the posterior division known as the *myelencephalon*. The cavity which extends through both is called the *fourth ventricle*.

THE SPINAL CORD AND ITS NEURAL CRESTS

The Cord. — As fast as the neural tube is formed by the fusion of the neural folds, its central canal tends to become compressed laterally and elongated dorso-ventrally. Its lateral walls also gradually thicken, and at the end of the second day these walls consist chiefly of two sorts of cells. First, there are elongated cells extending from the central canal out to its outer walls. These are the cells originally lining the canal, now known as *ependymal cells*, and their function is that of support. Secondly, among the ependymal cells and near the central canal are numerous rounded cells known as *germinal cells*. They later give rise to *neuroblasts* or primitive nerve cells, and also probably to more supporting elements termed *glia cells*. It has recently been claimed (Barron, '46) that some of the germinal (indifferent) cells are stimulated to become neuroblasts by contact with growing dendrites of other neuroblasts already partially differentiated.

The Neural Crests and Rudimentary Spinal Ganglia. — As indicated in the previous chapter, the neural crests when first formed are simply bands of cells which extend along the dorso-lateral walls of the neural tube, on either side between it and the ectoderm. As was also stated, these bands or crests are at first fused with one another dorsally. By the end of the second day, however, in the older (i.e., anterior) portion of the tube, this dorsal fusion has been obliterated. In this region there have also appeared in the crests successive enlargements, which presently become separated from one another to form a series of *rudimentary spinal ganglia*. There is one of these ganglia for each somite,

except for those of the head region, opposite whose somites the crests disappear. The spinal ganglia at this time contain both neuroblasts and indifferent cells.

THE CRANIAL GANGLIA

The neural crests of the head region anterior to the somites do not disappear, but also form enlargements which separate and take part in the formation of certain of the cranial ganglia. Parts of these ganglia, however, are placodal in origin, and surprisingly, according to some authors some of them even contain endodermal elements as indicated below. By the end of the second day the ganglionic rudiments are visible, beginning at the anterior end, in the following positions:

The V Nerve Ganglion.—The ganglion for the V or *trigeminal* nerve is somewhat anterior to the dorsal end of the first or mandibular arch. At the end of the second day it usually appears merely as a dark patch in this region (Fig. 176), but later (see third day) it acquires distinctly the form of an inverted Y. Apparently most, or all, of this ganglion is derived from crest material (Yntema, '44).

The VII and VIII Nerve Ganglia.—The ganglia for these nerves form a single mass, the *acustico-facialis* ganglion. It lies at this time just antero-ventral to the auditory sac (see below); i.e., it is above and slightly in front of the dorsal end of the second or hyoid arch. Though unlabeled, it is shown in Figure 176 in the position indicated. Jones, '42 has claimed that part of the VII ganglion is derived from the dorsal wall of the first visceral pouch, an unusual source of nerve tissue since the pouch is of course endoderm. Later study (Yntema, '44), however, seems to show that the origin is, as might be expected, partly crest and partly placode. The geniculate portion is thought to come from the placode, which, though closely associated with a pouch, is definitely not part of it, while the remainder of the facial nerve ganglionic complex is from the crest. The VIII ganglion appears to be entirely placodal.

The IX and X Nerve Ganglia.—The IX and X nerve ganglia arise together, but at the end of the second day they begin to become separated. The former, or *glossopharyngeal* ganglion, is then situated above the dorsal end of the third visceral arch while the latter, or *vagus ganglion*, lies above the ends of the fourth and fifth visceral arches. These ganglia are not visible in Figure 176. As to their sources, it appears that both contain some crest material, while it has again been claimed by both Winiwarter, '39 and Jones, '42 that material for the

petrosal portion of IX and the jugulare part of X are from the second and third visceral pouches respectively. It seems most probable, however, that, as in the case of the VII nerve ganglion, difficulty in separating the ectodermal and endodermal elements has led to error and that only "adjacent ectoderm," i.e., placode, is involved. A diagram of the location and form of the cranial ganglia viewed from above early on the second day is given in Figure 185.

ORGANS OF SPECIAL SENSE

THE EYE

The Optic Stalks, the Optic Cup and the Choroid Fissure. — The *optic vesicles*, it will be recalled, are hollow out-pushings from the fore-brain with which they remain connected by constricted regions known as the *optic stalks* (Fig. 186). These stalks are the so-called "optic nerves," though as will appear, the real optic nerves develop later. It is to be noted that the above constriction has occurred in such a manner that each stalk connects with its vesicle near the ventral side of the latter, rather than at its center. Invagination of the outer wall of the vesicle now occurs, obliterating its original cavity, and converting it into the two-layered *optic cup*, with the optic stalk attached to its ventral edge. The walls of the cup on either side of the point where the stalk is attached now grow outward, i.e., toward the ectoderm, but their ventral edges do not quite meet one another. Thus a fissure is left in the ventral side of the cup extending from its edge inward to the optic stalk. This, as in the Frog, is the *choroid fissure*. Meanwhile the rim of the cup bounding its aperture, the *pupil*, becomes slightly constricted. The invaginated or outer wall of the vesicle has now necessarily become

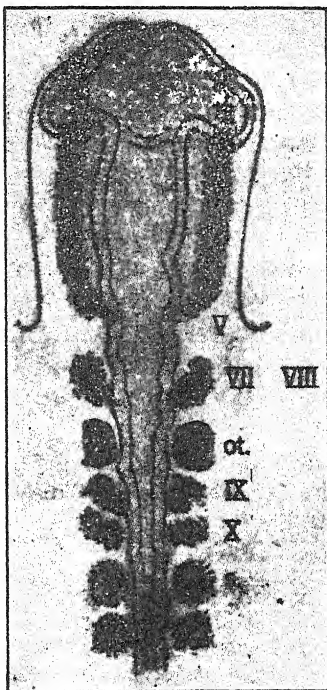


Fig. 185. — Diagram of the cephalic neural crest of a chick of about 12 somites. From Lillie (*Development of the Chick*). After Wilhelm His. *ot.* Auditory sac. *s.* Somite.

the inner wall of the cup, and will, therefore, be referred to as the inner wall in future discussion. It is the rudiment of the *nervous layer* of the *retina* (see Chapter 11).

The Development of the Lens. — Before the above invagination of each optic vesicle occurred, the vesicle had pushed out far enough to touch the surface ectoderm. When this happened, the ectoderm at the point of contact began to thicken, and when the invagination of the vesicle took place, this thickened ectodermal wall also invaginated. Thus a hollow thick-walled sac was formed resting just within the rim of the

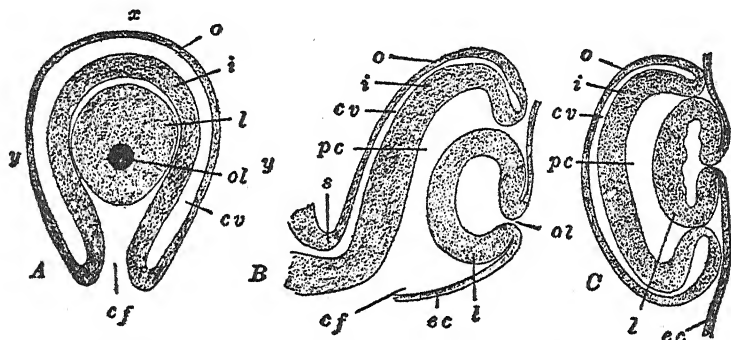


Fig. 186. — Diagrams of sections through the eye of the Chick embryo at the end of the second day. From Kellicott (*Chordate Development*). After Lillie. The dorsal margin is toward the top of the page in A and B. A. Eye as viewed directly. B. Vertical section through the line *x-y*, in A. C. Horizontal section through the line *y-y* in A. *cf*. Choroid fissure. *cv*. Cavity of primary optic vesicle. *ec*. Superficial ectoderm of head. *i*. Inner or nervous layer of the retina. *l*. Lens. *o*. Outer or pigmented layer of optic cup. *ol*. Opening of lens sac from surface of head. *pc*. Posterior (vitreous) chamber of eye. *s*. Optic stalk, continuous with the floor and lateral wall of the diencephalon.

optic cup. This is, of course, the rudiment of the *lens*; at the end of the second day it has not quite detached itself from the outer ectoderm.

As in the case of the Amphibian, it has been shown that the optic cup has the power to induce lens formation in ectoderm which would not otherwise form it. Thus optic vesicles or cups from embryos up to the 40-somite stage (fourth day) will induce lenses when transplanted to young hosts (primitive streak to eight somites). In a host older than four somites, however, the transplant will produce positive results only when implanted as far anterior as the potential head or neck region. In any case actual contact of the cup with the ectoderm seems necessary to effect induction. Also as in the Amphibian, the new lens may come from cells of the optic cup itself as well as from the host ectoderm (Alexander, '37), and the inductive process is a gradual one (McKeehan, '54).

THE EAR

The sensory part of the ear begins as a thickening of the ectoderm on the side of the head above and slightly posterior to the dorsal end of the hyoid arch. This thickening presently starts to invaginate, thus forming a depression — the *auditory pit*. During the second day the process of invagination continues, and is soon accompanied by an approximation of the anterior and posterior lips of the pit. Near the end of the second day the ventral lip also takes part in the closure by moving dorsally, and thus the pit is transformed into a small mouthed sac. It is the *auditory sac* or *otocyst* (Fig. 176).

THE URINOGENITAL SYSTEM

Because of their close connection in the adult, the excretory and reproductive systems are, as usual, considered under a common heading. Their development, however, is largely separate, and must, therefore, be so treated. Of the two systems, only certain parts of the excretory appear during the second day.

THE EXCRETORY SYSTEM

The excretory system of the Chick in common with that of other Amniota consists of three separate parts, the *pronephros*, *mesonephros*, and *metanephros*. These parts develop in the order named, and the first two have largely disappeared by the close of embryonic life; only the last remains functional as the permanent excretory organ of the adult. During the second day the pronephros develops, and near its close the mesonephros has just begun to appear.

The Pronephros. — The pronephros is vestigial in character, and only appears typically from the tenth to the fifteenth somites. Rudiments of it, however, are sometimes found as far forward as the fifth somite. In the more posterior region indicated, its development is as follows:

The Pronephric Tubules. — In the dorso-lateral portion of the nephrotome opposite the posterior end of each somite a thickening occurs, and from it a cord of cells grows outward and upward for a short distance (Fig. 187, *pr'n.* 1). At the same time the nephrotome becomes detached from the somite. These lateral outgrowths are termed the *pronephric tubules*, though they usually do not acquire any lumen. Some-

times, however, a slight lumen is present in the proximal part of the tubule (Fig. 187, *pr'n. 2*), and it opens into the coelom as a rudimentary *nephrostome*. It is also said that degenerate *glomeruli* (or more properly *glomi*) sometimes develop later on the coelomic wall opposite the nephrostomal mouths (Lillie).

The Pronephric and Wolfian Ducts. — The distal part of each of the above cell cords or "tubules" presently bends posteriorly and grows in

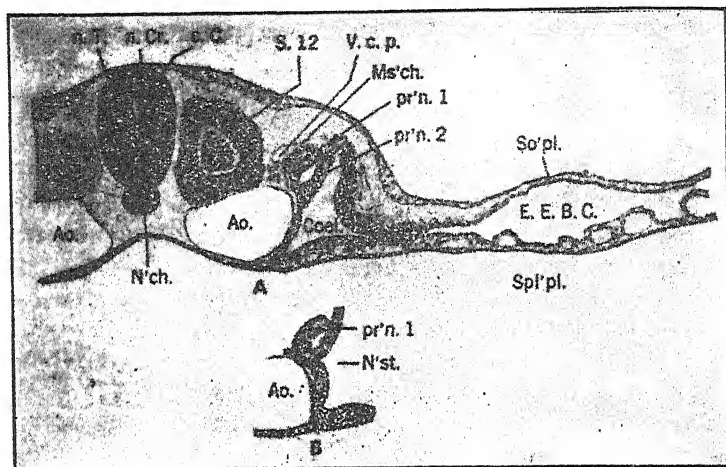


Fig. 187. — A. Transverse section through the twelfth somite of a 16s embryo. From Lillie (*Development of the Chick*). B. Three sections behind A to show the nephrostome of the same pronephric tubule.

Ao. Aorta. c.C. Central canal. Coel. Coelom. E.E.B.C. Extra-embryonic coelom (exocoelom). Ms'ch. Mesenchyme. N'ch. Notochord. n.Cr. Neural crest. N'st. Nephrostome. n.T. Neural tube. *pr'n. 1, 2*. Distal and proximal divisions of pronephric tubule. S.12. Twelfth somite. So'pl. Somatopleure. Spl'pl. Splanchnopleure. V.c.p. Posterior cardinal vein.

this direction until it comes in contact with the tubule following it. In this manner, a continuous backwardly directed cord of cells is formed which connects with each successive tubule. Finally, the bent portion of the last cell cord continues to grow posteriorly between the nephrostomal mass and the body wall. As will appear subsequently, the anterior end of this backward growing rod of cells is the rudiment of the *pronephric duct*, and its more posterior portion, the rudiment of the *mesonephric* or *Wolfian duct*. Before the end of the second day, indeed, the anterior or pronephric section of the rod has acquired a lumen, thus becoming a real duct.

The Mesonephros. — The mesonephros corresponds to the organ of the same name which functions as the permanent excretory organ of the Frog. In the Chick, however, as indicated above, this excretory function continues only during a part of embryonic life. The anterior end of the mesonephros slightly overlaps the posterior end of the pronephric region, but its development here is rudimentary, the organ acquiring its typical form only from the twentieth to the thirtieth somites. During the close of the second day it begins to appear in the following manner, development progressing posteriorly.

The Primary Mesonephric Tubules. — The nephrotome in the region indicated becomes separated both from the somites and the lateral plate. It then lies just ventro-medially to the rod of cells which is to become the Wolffian duct. Above this duct the posterior cardinal vein presently appears, while between the nephrotome and the median line of the embryo runs the dorsal aorta. The nephrotome is thus between the aorta and the future Wolffian duct (Fig. 174). Presently in the neighborhood of each somite, there appear in this nephrotomal band two or more spherical condensations. Then beginning at the anterior end of the band each of these condensed spheres starts to acquire a cavity, each vesicle thus formed being the rudiment of a *mesonephric tubule* and a *Malpighian body*. The more ventral spheres in each somite are the first thus to become vesicular, and they are the rudiments of the so-called *primary mesonephric tubules* as distinguished from the others. (See next chapter, Fig. 207.)

AMNION AND OTHER EXTRA-EMBRYONIC STRUCTURES

From the embryological point of view all Vertebrates belong to one of two classes; i.e., the *Anamniota* or the *Amniota*. The former group includes Amphibians and Fishes, while the latter includes Reptiles, Birds, and Mammals. The Amniota, as the name implies, are those which possess an amnion, while the Anamniota are those which lack it. Amphioxus, the Frog, and Fish have been studied as representatives of the latter class, and we are now studying the Chick as an example of the former or Amniote group. The amnion begins to form on the second day of the Chick's incubation, but is not completed until about the fourth day. In order to make the structure of this organ more clear, however, it seems best to describe its entire development, together with that of certain other extra-embryonic organs and membranes.

THE AMNION IN PROCESS OF DEVELOPMENT

Development during the Second Day. — During the second day a fold in the blastoderm occurs just in front of the head of the embryo in the region of the proamnion. Since there is as yet no mesoderm in this region, the fold at first contains only ectoderm and endoderm. Presently, however, the mesoderm extends into this vicinity, and here, as elsewhere, is split into the extra-embryonic extensions of the somatic

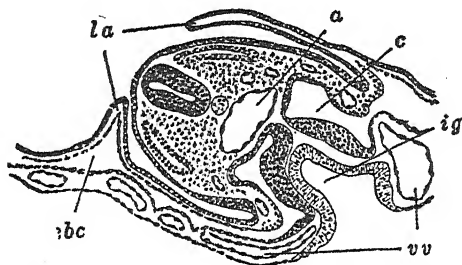


Fig. 188. — Diagrammatic transverse section through the region where the gut is open out over the yolk (yolk-stalk umbilicus), in a Chick of about 48 hours (about 28 pairs of somites). From Kellicott (*Chordate Development*). After Duval.
a Dorsal aorta. *c* Coelom. *ebc*: exocoelom. *ig*. Intestinal groove. *la*. Lateral folds of amnion. *vv*. Vitelline vein.

and splanchnic layers with the extra-embryonic coelomic space between them; both these layers then become involved in the fold. The splanchnic layer together with the endoderm, however, is soon withdrawn to the surface of the yolk, while the somatic layer and the extra-embryonic ectoderm which covers it constitute the two permanent layers of the *amniotic head fold*.

The embryo has now begun to sink somewhat into the surface of the yolk, and as it does so the amniotic fold gradually grows back over it. This backward growth is also accompanied by the development of *lateral amniotic folds* extending posteriorly on either side. By the end of the second day the embryo has been covered over in this manner almost as far back as the vitelline arteries (Figs. 176 and 188). The latter figure shows a cross section through a region where the folds have not yet quite covered the embryo.

Development during the Third Day. — About the end of the second day, or the beginning of the third, another fold appears at the posterior end of the embryo, and grows forward toward the head fold. This is the *amniotic tail fold*, which soon becomes coextensive upon either side with the posterior ends of the lateral amniotic folds. It is similar to the corresponding head fold except that from the first it contains only ectoderm and somatic mesoderm. Since the anterior portion of the amnion starts earlier and grows rapidly, the point at which the converg-

ing folds finally meet and fuse is quite near the posterior end of the animal. The oval opening existing above the Chick previous to the closure is the *amniotic umbilicus*.

Development during the Fourth Day. — The end of the third, or beginning of the fourth day, marks the meeting and fusion of the amniotic folds at the center of the amniotic umbilicus. The embryo has by this time turned upon its left side throughout the greater part of its

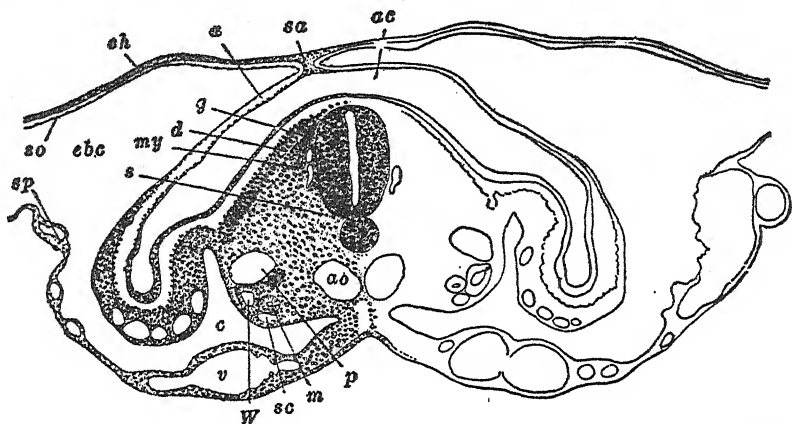


Fig. 189. — Transverse section of Chick embryo with 35 pairs of somites (about 72 hours), passing through the region of the twenty-third somite. From Kellicott (*Chordate Development*). After Lillie.

a. Amnion. ac. Amniotic cavity. ao. Dorsal aorta. c. Embryonic coelom. ch. Chorion. d. Dermatome. ebc. exocoelom. g. Rudiment of spinal ganglion. m. Mesonephric tubule. my. Myotome. p. Posterior cardinal vein. s. Sclerotome. sa. Sero-amniotic connection. sc. Subcardinal vein. so. Somatic mesoderm. sp. Splanchnic mesoderm. v. Vitelline artery. W. Wolffian duct.

length, and inasmuch as the folds do not turn with it, the closure occurs not above its back, but above its right side. It also follows from this, that the fold of the left side covers the back of the embryo as well as a part of the right side. The amnion may now be said to be complete.

THE COMPLETED AMNION AND RELATED PARTS

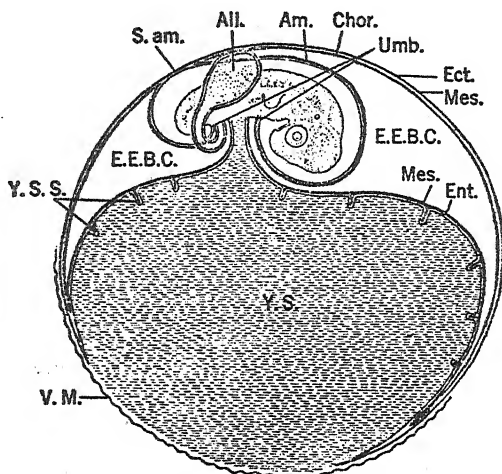
The Amnion and Amniotic Cavity. — It is obvious that the amniotic folds, like any other folds, must be composed of two main parts, each part being continuous with the other at the crest of the fold. It is also obvious that one of these parts, i.e., the inner or lower one, lies everywhere next to the embryo. When fusion occurs, therefore, this inner

part will become continuous, completely bounding a new cavity which surrounds the embryo at every point except for a restricted region on its ventral side (see below under somatic umbilicus). This continuous inner membrane is the *amnion*, and the cavity thus formed is the *amniotic cavity*. Moreover, inasmuch as the folds involve both ectoderm and mesoderm, the inner membrane or amnion must likewise consist of ectoderm and mesoderm, the former lining the amniotic cavity and the latter forming a coat outside the lining (Figs. 189 and 190).

The Chorion.—At the fusion of the folds the outer part, like the inner, necessarily becomes continuous. Likewise, it too consists of both ectoderm and mesoderm, but in this case, the ectoderm will lie outside and the mesoderm inside, i.e., toward the amnion. The outer membrane thus formed is called the *chorion*, *serosa* or *false amnion*. Between it and the inner membrane or true amnion, there is naturally the same space which separated the inner and outer parts of the amniotic folds, i.e., the extra-embryonic coelom or *exocoelom*. This relationship will be made clear by reference to Figure 190. It may be mentioned incidentally in this connection that this exocoelomic space eventually becomes filled by an important sac-like organ (*allantois*) whose origin and structure will be described below.

The Sero-Amniotic Connection.—It has been implied that the extra-embryonic coelom, with whatever may occupy it, everywhere separates the amniotic membrane from the chorionic membrane. This is true except at one point. At the point of final fusion of the amniotic folds, i.e., the amniotic umbilicus, the coelomic space is interrupted by a small area of mesoderm which persists, and serves to unite the above membranes. It is called the *sero-amniotic connection* (Figs. 189 and 190).

The Amniotic Fluid.—Shortly after the completion of the amniotic cavity, fluid begins to accumulate within it. Thus the embryo is soon practically surrounded by a liquid cushion which protects it from pressure by its membranes and rigid shell. This is the *amniotic fluid*. Presently, about the fifth day, muscle fibers develop in the mesoderm of the amnion and begin to send waves of contraction over it. This causes a gentle rocking of the embryo, and is apparently instrumental in preventing its adhesion to the various embryonic membranes. It may also help to obviate the stagnation of blood in the vessels, a condition which might tend to occur on account of the pressure from the growing organs.



Figs. 190, 191, 192. — Diagrams of the relations of the extra-embryonic membranes in the Chick. Figures and description from Lillie (*Development of the Chick*). The ectoderm and endoderm are represented by plain lines; the mesoderm by a cross-hatched line or band. The yolk-sac is represented by broken parallel lines. In Fig. 190 the allantois is represented as a sac. In Figs. 191 and 192, where it is supposed to be seen in section, its cavity is represented by unbroken parallel lines. The stalk of the allantois is exaggerated in all the diagrams to bring out its connection with the embryo.

Fig. 190. — Fourth day of incubation. The embryo is surrounded by the amnion which arises from the somatic umbilicus, *Umb.*, in front and behind; the sero-amniotic connection, *S.am.*, is represented above the tail of the embryo; it consists at this time of a fusion of the ectoderm of the amnion and chorion. The allantois, *All.*, is represented as a sac, the stalk of which enters the umbilicus behind the yolk-stalk; the allantois lies in the extra-embryonic body-cavity (exocoelom), and its mesodermal layer is fused with the corresponding layer of the chorion above the embryo. The septa of the yolk-sac, *Y.S.S.*, are represented at an early stage. The splitting of the mesoderm has progressed beyond the equator of the yolk-sac, and the undivided portion is slightly thickened to form the beginning of the connective-tissue ring that surrounds the yolk-sac umbilicus. The ectoderm and endoderm meet in the zone of junction, beyond which the ectoderm is continued a short distance. The vitelline membrane, *V.M.*, is ruptured, but still covers the yolk in the neighborhood of the yolk-sac umbilicus. The albumen is not represented in this figure. (For complete explanation of lettering see Fig. 192.)

THE SOMATIC UMBILICUS, THE YOLK-STALK, AND THE YOLK-SAC

Though they are not a part of the amnion, it seems best to include in connection with its description an account of these structures which, to some extent, develop with it.

The Somatic Umbilicus. — During the formation of the amnion, the gradual separation of the embryo from the yolk has been progressing. This has been accomplished by the steady in-pushing of the ventral portions of the head, tail, and lateral folds (*limiting sulci*) beneath the body of the growing Chick. The result is that by the time the amnion is completed, these folds have approached one another quite closely, though without coming into contact. In this manner they give rise to a short, thick, hollow stalk which connects the embryo with the yolk-sac and its extra-embryonic membranes. The outermost wall of this stalk is continuous with that of the amnion, and is, therefore, composed of ectoderm and somatic mesoderm: for this reason, this outer wall is referred to as the *somatic umbilicus* (Fig. 190).

The Yolk-Stalk. — Within this wall and surrounding the inner wall of the stalk, is a space continuous externally with the extra-embryonic coelom and internally with the coelom of the embryo itself. Finally, the inner wall of the stalk consists of splanchnic mesoderm and endoderm. It is known as the *yolk-stalk*, but is really merely an inner tube of the somatic umbilicus separated from it by coelomic space.

The Yolk-Sac. — The wall of the yolk-stalk is coextensive within the embryo with the wall of the gut, and externally with the layer of endoderm and the splanchnic mesoderm which overlies the yolk. This layer is continually growing out around the yolk, and at its outermost border, i.e., the region of the zone of junction, the endodermal portion of it becomes continuous with the chorion which overlies it. Thus by means of the extension of these layers the yolk is gradually enclosed in a covering, whose inner layer of splanchnic mesoderm and endoderm constitutes the *yolk-sac*, attached to the embryo by means of the yolk-stalk. Upon the ninth day of incubation this sac has become virtually complete, save at a point on the side of the yolk postero-ventral to the body of the Chick, where an opening remains, known as the *yolk-sac umbilicus*. This opening, however, is finally closed about the seventeenth day by a solid mass of tissue. It may be recalled in this connection that the rim of the blastoderm, which has thus overgrown the yolk, was previously homologized with the lip of a very extended blastopore,

the true blastopore (primitive streak) having been separated from the remainder of the rim during gastrulation. Hence upon this basis it is possible to consider the uncovered yolk mass as a sort of very large secondary, or *yolk-blastopore*, the latter term being really only another name for the yolk-sac umbilicus. A somewhat similar separate blastopore, it may be noted, also occurs in the development of the Elasmobranchs (i.e., the cartilaginous or non-bony fishes) in which the term yolk-blastopore is regularly applied to it.

On the basis of this description, it is clear that beyond the boundaries of the amnion the chorion is really nothing more than the uppermost layer of the blastoderm. It is to be noted, however, that this upper layer consisting of ectoderm and somatic mesoderm is soon separated from the lower layer composed of splanchnic mesoderm and endoderm by the extra-embryonic coelom. Furthermore, this space presently becomes occupied by another extra-embryonic organ (allantois), to be described below. Finally it must also be mentioned that early in its development, the lower layer, just indicated, i.e., the real yolk-sac layer, consisting of endoderm and splanchnic mesoderm, becomes covered internally with deep folds, the *yolk-sac septa*, which gradually press downward into the yolk. These septa in common with the remainder of the yolk-sac endoderm in the area vasculosa, contain glandular and absorbing cells which digest the yolk *in situ* before passing it into the blood vessels. Thus though a slight lumen exists in the yolk-stalk connecting the inside of the yolk-sac with the enteric canal, no yolk appears to pass into the embryo through this lumen. Abnormally high or low temperatures during incubation, e.g., 39.5° C and 34.5° C, appear to slow up the process of absorption of both yolk and albumen (Romanoff, '43).

THE ALLANTOIS

Another extremely important extra-embryonic organ possessed in some degree by all Amniota is the *allantois*, and it will be found convenient to consider its entire history also at this time.

Its Early Development. — The allantois starts in the form of an out-pushing from the ventral wall of the hind-gut (Fig. 193). This is scarcely visible before the beginning of the third day, and was, therefore, not referred to in the foregoing description of the alimentary tract. This out-pushing naturally involves the endoderm and the mesodermal ventral mesentery which occurs in this region. Thus the sac which is presently formed possesses an inner endodermal and an outer mesoder-

manner, the above ramifications of the blood vessels are brought very near to the shell, through which an exchange of gases is possible. Thus the allantois serves as an organ of respiration for the Chick during embryonic life. Its cavity also acts as a receptacle for the waste products of

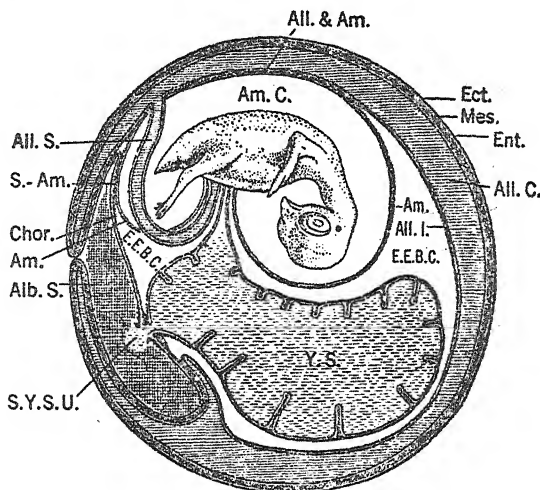


Fig. 192. — Twelfth day of incubation. The conditions are more advanced than those represented in Fig. 191. The albumen-sac is closing; its connection with the cavity of the amnion by way of the sero-amniotic connection will be obvious. The inner wall of the allantois has fused extensively with the amnion. The umbilicus of the yolk-sac is much reduced, and some yolk protrudes into the albumen (sac of the yolk-sac umbilicus, transitory structure soon drawn into the yolk-sac proper).

Alb. Albumen. *Alb.S.* Albumen-sac. *All.* Allantois. *All.I.* Inner wall of allantois. *All.C.* Allantoic cavity. *All.S.* Allantoic stalk or neck. *All. + Am.* Fusion of allantois and amnion. *Am.* Amnion. *Am.C.* Amniotic cavity. *Chor.* Chorion. *C.T.R.* Connective-tissue ring. *Ect.* Ectoderm. *E.E.B.C.* Exocoelom (extra-embryonic body-cavity). *Ent.* Endoderm. *Mes.* Mesoderm. *S.-Am.* Sero-amniotic connection. *S.Y.S.U.* Sac of the yolk-sac umbilicus. *Umb.* Umbilicus. (somatic). *V.M.* Vitelline membrane. *Y.S.* Yolk-sac. *Y.S.S.* Septa of yolk-sac.

metabolism, which are conveyed thither through the allantoic stalk from the region of the cloaca. It is thus to be noted that this organ is homologous not only in method of origin, but also partly in function with the urinary bladder of the Frog. The latter, however, of course never extends outside of the coelomic cavity, and though it may or may not be endodermal, the allantois is certainly so.

The Later Development of the Allantois and the Formation of the Albumen-Sac. — Meanwhile the albumen is becoming concentrated on the side of the egg next to the yolk-sac umbilicus, and by the ninth or tenth day has become very much condensed. Concurrently the real yolk-sac layer, together with the chorion, has grown around the yolk so that the edges of the over-growth have more than kept in contact with the receding albumen. They have in fact thrust themselves in between it and the yolk, so that the albumen is bounded upon its inner side by a layer of chorion. At the same time, save postero-dorsally in the region of the sero-amniotic connection, the allantois has been following this over-growth of the yolk-sac layer and chorion; it lies between these two layers in the exocoelom, and its walls are fused respectively with the chorionic layer and that of the yolk-sac. Thus as the latter layers push in between the yolk and the albumen to close the yolk-sac umbilicus, they are accompanied, except postero-dorsally, by the allantois. Ventro-laterally a fold of the chorion presently pushes its way around the outside of the albumen between it and the shell membrane. Here too, moreover, between the two layers of the chorionic fold there follows an outer fold of the allantois. Meanwhile in the postero-dorsal region, as already suggested, the expansion of this organ is obstructed by the sero-amniotic connection. At this point, therefore, it pushes up over this connection, carrying the chorion before it. Thus this dorsal fold, consisting of a layer of chorion and allantoic wall, comes down between the albumen and shell membrane to meet the similarly constituted ventro-lateral folds already described. Hence, at ten days the albumen at the yolk-sac umbilicus is surrounded by a double layer of fused chorionic and allantoic tissue, the *albumen-sac*. There is just one region in the wall of the sac, however, where all of these layers are not present. This is a small area on its internal dorsal side where the allantois could not extend because of the sero-amniotic connection. There, therefore, the wall consists only of chorion, and at one point of the connection itself (Figs. 191, 192). A perforation appears in this connection, and on the twelfth day some albumen enters the amniotic cavity. The remainder of the albumen is absorbed, and the albumen-sac together with the yolk-sac is drawn within the embryo just previous to hatching. According to Randles and Romanoff, '50, a periodic turning of the egg is necessary if all these events are to be accomplished normally at the times indicated. Hatching is apparently aided by the contraction of the muscular walls of the allantois and by the muscles of the somatic umbilicus (see also Fig. 193).

SUMMARY OF THE CONDITION AT THE END OF THE SECOND DAY OF INCUBATION

I. GENERAL APPEARANCE

The cranial flexure has been initiated, and has brought the fore-brain to a point where it almost touches the heart, and the mid-brain faces anteriorly. The cervical flexure is also evident in the region of hind-brain and trunk. In correlation with these flexures lateral rotation has started so that the embryo lies on its side as far back as the 13th somite.

II. THE SOMITES

There are approximately 27 somites, in which the *myotomes* and *cutis plates* have begun to differentiate, together with the mesenchymatous rudiment of the *sclerotome*.

III. THE FORE-GUT

In the fore-gut the *stomodaeum* is formed, and in connection with it Rathke's pocket, a part of the future *hypophysis*, is beginning to appear. Four pairs of *visceral pouches* and five pairs of *arches* have begun to develop, and the first pair of pouches have acquired openings to the exterior. The rudiments of the *thyroid*, the *respiratory system*, and the *liver* are also present.

IV. THE MID-GUT

This is but slightly developed, although the lateral folds are beginning to mark it off from the extra-embryonic archenteron.

V. THE HIND-GUT

The hind-gut has begun to form and its posterior end has fused with the ectoderm to form the *anal plate* or *cloacal membrane*. In connection with it there has also arisen the *ventral mesentery*.

VI. THE CIRCULATORY SYSTEM

The Heart. — A bent *tubular heart* has been developed, lined by *endothelium* and covered with a *myocardium*. The regions of the *atria*, the *ventricles*, and the *bulbus* and *truncus arteriosus* are indicated, and pulsation has been initiated.

The Arteries.—The *dorsal aortae* are in evidence. Also the *ventral aorta* has appeared and become incorporated into the truncus. The first pair of aortic arches are in process of formation, and the second and third aortic arches are completed. The *vitelline arteries* have appeared.

The Veins.—The *anterior* and *posterior cardinals*, the *sinus venosus*, the *ductus venosus*, and the *ducts of Cuvier* have been developed. In connection with the latter the septa known as the *lateral mesocardia*

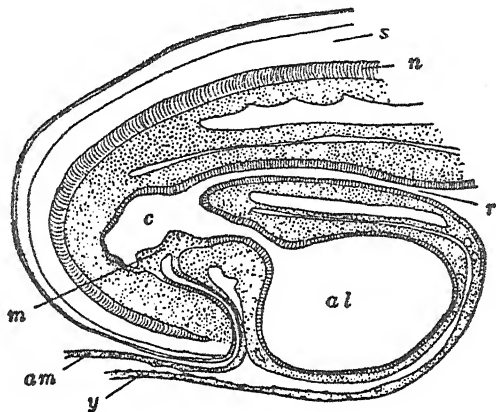


Fig. 193. — Median sagittal section through posterior end of four-day chick. From Kellicott (*Chordate Development*). After Gasser (Maurer).

al. Allantois. *am.* Amnion (tail-fold). *c.* Cloaca. *m.* Cloacal membrane. *n.* Notochord. *r.* Rectum. *s.* Spinal cord. *y.* Wall of yolk-sac (endoderm and splanchnic mesoderm).

have also been formed. Outside the embryo the *anterior vitelline veins* have arisen, and with them the rudiments of the *lateral vitelline veins*. The *sinus terminalis* has become complete.

VII. THE NERVOUS SYSTEM

The Brain and the Cranial Ganglia.—As indicated under external appearance the *cranial* and *cervical flexures* have become well marked. The *fore-brain*, *mid-brain* and *hind-brain* are now clearly indicated, and within the first main division certain parts are apparent, as follows: The outgrowth of the *optic stalks* is well advanced, and there may also be evident the rudiments of the *optic chiasma*, the *optic recess*, the *cerebral hemispheres*, the *infundibulum*, and some other minor structures. The roof of the mid-brain is becoming prominently arched.

The *cranial ganglionic rudiments* of the V, VII and VIII, and IX and X nerves are visible, and the latter pair are beginning to separate.

The Spinal Cord and Ganglia. — The spinal cord has become thick-walled laterally, and has developed *ependymal* and *germinal* cells. The *neural crests* are segmenting to form the spinal *ganglia*.

VIII. THE ORGANS OF SPECIAL SENSE

The *optic vesicles* have become invaginated to form the *optic cups*, and the external ectoderm opposite each cup has invaginated in the process of forming a *lens*. In connection with the *ear*, the auditory portion of the ectoderm has become invaginated to form the *auditory sac*.

IX. THE URINOGENITAL SYSTEM

Only the embryonic parts of the excretory portion of this system appear during the second day. These are the *pronephros*, including the *Wolffian duct*, and the rudiments of the *mesonephros*. These rudiments consist of concentrations of nephrogenous tissue, some of which are beginning to become vesicular in the formation of the *mesonephric tubules* and the *Malpighian bodies*.

X. THE AMNION

This extra-embryonic organ begins its development on the second day with the appearance of the *amniotic head fold*, the *amniotic lateral folds*, and sometimes an indication of the *amniotic tail fold*.

The complete development of the *amnion*, the *chorion*, the *allantois*, and the *yolk-sac* is described in this chapter.



THE CHICK: DEVELOPMENT DURING THE THIRD DAY OF INCUBATION

GENERAL APPEARANCE

FLEXURES AND TORSION

THE embryo has of course increased somewhat in size, but the most obvious changes concern the flexures. The *cranial flexure* is somewhat more marked, while the *cervical flexure* has greatly increased, so that the region of the hind-brain, rather than the mid-brain is now the most anterior part of the embryo. By the close of this day also a new curvature has become evident at the posterior end. It involves mainly the tail, and is called the *caudal flexure*. Between this flexure and the cervical flexure the back of the embryo is temporarily somewhat bent in a ventral direction, i.e., opposite to the other curvatures. This is because of the broad attachment to the yolk which still extends throughout the middle region and tends to draw this part of the embryo ventrad (Fig. 200). Accompanying these increases in flexure the *lateral rotation* has progressed posteriorly until by the end of the day the embryo is on its side about as far back as the twenty-first somite.

LIMB BUDS

The limb buds become clearly visible by the end of the third day, and appear as broad swellings on either side of the embryo. The anterior buds extend from about the fifteenth to the twentieth somite, and the posterior buds from about the twenty-seventh to the thirty-third somite.

THE SOMITES

During the third day the number of pairs of somites increases to about 36. The newer posterior somites when first formed are in the same condition as were those which are now anterior, and are destined to go

through the same process of development. Meanwhile, the more advanced anterior members of the series do not greatly change except for further modifications along the lines already indicated on the second day. These modifications are as follows:

Each myotome or muscle plate continues to grow down along the inside of its respective cutis plate, until in the most mature somites it reaches the ventral end of the cutis plate and fuses with it. In this manner a complete double layer of cells arises. In the inner layer or muscle plate thus formed, the cells or myoblasts presently begin to become spindle-shaped, reaching from the anterior to the posterior walls of each myotome. These are mostly rudiments of dorsal voluntary muscles. Somewhat later on the third day the outer or cutis plates of somites which have reached this stage begin to break up into mesenchyme, which wanders outward toward the ectodermal wall. There it eventually gives rise to the *dermis* of the dorsal region, that of the lateral and ventral parts being derived from the adjacent somatopleure (Murray, '28).

The sclerotomal mesenchyme continues to collect about the notochord and the sides of the nerve cord.

THE ALIMENTARY TRACT

THE FORE-GUT

The Oral Cavity.— During the third day, the oral plate breaks through, placing the stomodaeum in direct communication with the pharynx (Fig. 204). The region in which the digestive tract opens to the exterior anteriorly is thus partly stomodaeal and partly pharyngeal. It is called the *oral cavity*.

The Hypophysis or Pituitary Body.— It will be recalled that at 24 hours a hollow diverticulum called Rathke's pocket was extending forward from the roof of the stomodaeum toward the floor of the diencephalon in the vicinity of the infundibulum. At about the 30-somite stage it has nearly reached the latter organ (Fig. 204), and shortly its end begins to broaden out and become branched. Finally, near the end of the incubation period, these branches have become a mass of tubular tissue well supplied with blood vessels. This glandular mass then loses all connection with the oral epithelium from which it arose, and becomes firmly attached to the infundibulum. In this manner the original Rathke's pocket comes to constitute the *anterior part* of the *hypophysis* or *pituitary body*, while the infundibulum becomes the *posterior part*

and *stalk* of that organ. Experimental work has shown that the outgrowth of Rathke's pocket is originally induced by the presence of the infundibulum, and that both structures influence one another in the normal development of the completed organ (Hillemann, '43). It may be recalled that this same relationship is true in the Frog, except that there the homologue of Rathke's pocket is merely a strand of cells.

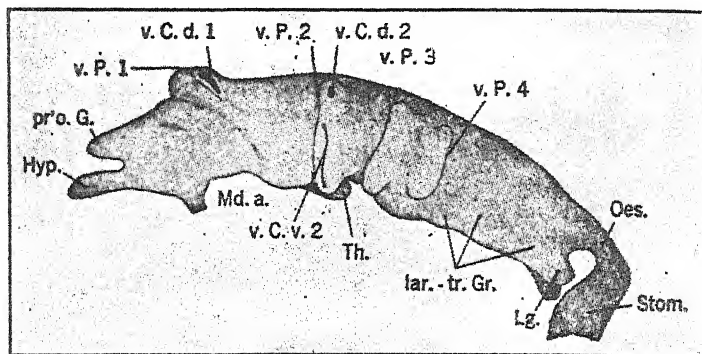


Fig. 194. — Reconstruction of the fore-gut of a Chick of 72 hours. From Lillie (*Development of the Chick*). After Kastschenko.

Hyp. Rathke's pocket, rudiment of anterior hypophysis. *lar.-tr.Gr.* Laryngotracheal groove. *Lg.* Lung. *Md.a.* Mandibular arch. *Oes.* Oesophagus. *pr.o.G.* Preoral gut. *Stom.* Stomach. *Th.* Thyroid. *v.C.d. 1, 2.* Dorsal division of the first and second visceral clefts. *v.C.v. 2.* Ventral division of the second visceral cleft. *v.P. 1, 2, 3, 4.* First, second, third, and fourth visceral pouches.

The Visceral Pouches and Arches.

The Pouches. — It will be remembered that during the second day four pairs of visceral pouches had appeared; the first three had reached the ectoderm, and each member of the first pair had acquired a cleft opening to the outside. During the third day the first pair of pouches retain their openings, while each member of the second pair develops a short dorsal and a long ventral cleft, corresponding to the points of fusion between ectoderm and endoderm described in the preceding chapter. The members of the fourth pair of pouches now acquire connections with the ectoderm at their dorsal ends, but never develop any clefts (Fig. 194).

The Arches. — The visceral arches undergo no special change on the third day, except the development in some of them of the aortic blood vessels (arches) which will be described below.

The Thyroid. — During the third day, the rudiment of the thyroid which was last described as a slight depression in the floor of the phar-

ynx, continues to evaginate. By means of this process, the end of the third day finds the above depression transformed into a wide-mouthed sac. Figure 195 shows in cross section this and other structures indicated above.

The Laryngotracheal Groove and Lung Primordia.—At the end of the second day a shallow longitudinal groove with a pair of

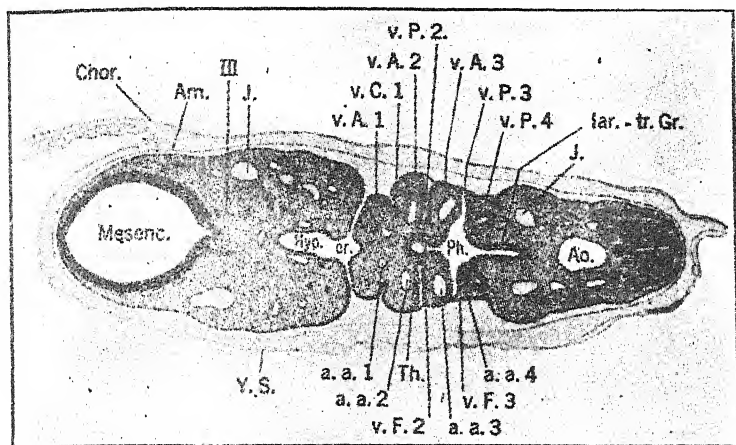


Fig. 195.—Frontal section through the pharynx of a 35 somite embryo. From Lillie (*Development of the Chick*).

a.a. 1, 2, 3, 4. First, second, third, fourth aortic arches. Hyp. Rathke's pocket, rudiment of anterior hypophysis. J. Jugular vein. lar.-tr. Gr. Laryngotracheal groove (post branchial pharynx). or. Oral cavity. Ph. Pharynx. v.A. 1, 2, 3. First, second, third visceral arches. v.C. 1. First visceral cleft. v.F. 2, 3. Second and third visceral furrows. v.P. 2, 3, 4. Second, third, fourth visceral pouches. III. Third cranial nerve.

postero-lateral expansions had appeared in the floor of the pharynx just caudal to the visceral pouches, indicating the beginning of the respiratory system. This groove now becomes much narrower and deeper, and is called the *laryngotracheal groove*. Also its postero-lateral expansions develop into tubelike outgrowths which, as previously indicated, are then ordinarily termed the *lung primordia*. Strictly speaking, however, they really represent, not only the beginnings of the lungs, but also of the *bronchi*, i.e., the entire respiratory system.

The Esophagus and the Stomach.—By the end of the third day the *esophagus* is represented by an abrupt narrowing of the fore-gut immediately posterior to the pharynx. The narrowed portion leads into a slightly dilated region just anterior to the liver rudiment, and this dilation is the beginning of the *stomach*, i.e., the proventriculus and gizzard (see the fifth day).

The Liver. — At the end of the second day the liver was represented by two anteriorly directed diverticula from the region of the anterior intestinal portal; the more anterior of these had extended far enough forward to overlie slightly the point of union of the vitelline veins. During the third day, these diverticula grow somewhat further forward, the anterior member of the pair along the left dorsal side of the ductus venosus, and the posterior member along its right ventral side. Both

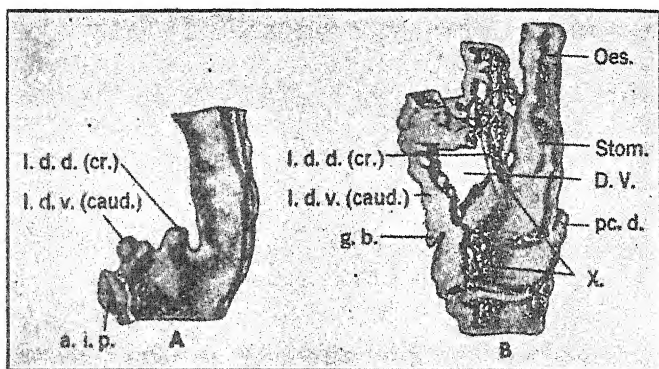


Fig. 196. — Reconstructions of the liver diverticula of the Chick. From Lillie (*Development of the Chick*). After Hammar.

A. On the third day of incubation; from the left side; the diverticular arise from the anterior intestinal portal.

B. Beginning of the fourth day; from the left side.

a.i.p. Anterior intestinal portal. *D.V.* Indicates position of ductus venosus. *g.b.* Gall bladder. *l.d.d.(cr.)*. Dorsal or cranial liver diverticulum. *l.d.v.(caud.)*. Ventral or caudal liver diverticulum. *pc.d.* Dorsal pancreas. *X.* Marks the depression in the floor of the duodenum from which the common bile duct is formed.

now also branch profusely, the branches spreading around the ductus venosus and anastomosing freely with one another. At the same time capillaries from the ductus venosus begin to develop among the interstices of these anastomosing branches; this is the beginning of the main body of the liver.

The Bile Ducts. — In the meantime, the intestinal portal has, of course, moved backward beyond the point of origin of the diverticula. This lengthens the gut and leaves these diverticula attached to its ventral side at their points of origin. The parts of the diverticula between the region of their anastomosis and the points of attachment to the gut are of the nature of short tubes, the rudiments of the future *bile ducts*. Presently the floor of the gut comprising the region where these ducts enter it becomes depressed, and then drawn out so as to form a common

duct into which the two original ducts empty. This common duct is called the *ductus choledochus*, and is a temporary structure (Fig. 196).

The Gall Bladder. — While the above processes have been going on, the *gall bladder* has arisen as a posterior evagination from the posterior liver diverticulum. As the latter then grows forward, its attachment to the gall bladder is drawn out to form the *cystic bile duct*.

All of these hepatic structures it should be noted are covered by the splanchnic mesoderm of the ventral mesentery within which they have developed. This mesentery, here termed the *gastro-hepatic ligament*, serves permanently to attach the whole mass to the gut and stomach.

The Pancreas. — This organ first appears on the third day as a thickening on the dorsal wall of the intestine within the dorsal mesentery about opposite the posterior liver diverticulum. The rudiment thus indicated gives rise to only about a third of the entire organ whose further development will be described as it occurs (Fig. 196).

THE MID-GUT

There is no great change in the mid-gut region during the third day except that it becomes more clearly marked off as the lateral folds continue to press in toward one another.

THE HIND-GUT

The Postanal Gut. — It will be recalled that at the close of the second day the ectoderm had taken so slight a part in the tail fold that the anal plate retained a dorsal position. On the third day, however, the fold becomes more marked, and soon takes on the character of a posterior outgrowth, which is at first anterior to the anal plate. This outgrowth is the *tail bud*. As its development progresses it becomes first postero-dorsal, and then by turning downward postero-ventral, to the anal plate, which itself becomes ventral instead of dorsal (Figs. 197, 198). Also as a result of this process there is drawn out into the bud an extension of the hind-gut, constituting a temporary structure known as the *postanal gut* (Fig. 197).

The Allantois. — The most important structure to appear in connection with the hind-gut during early embryonic life is the allantois. The rudiment of this organ is usually indicated at about the beginning of the third day. The method of its development and its final structure have been described above (Figs. 190, 193). In connection with the diagrams presented in Figure 198, however, a further word about its early origin should be said. These diagrams represent the behavior of this re-

gion as described in the text, and according to Gruenwald ('41). It must be added, however, that in spite of the fact that there is apparent agreement regarding the movements which are taking place, Gruenwald puts a somewhat different interpretation on them than do certain other au-

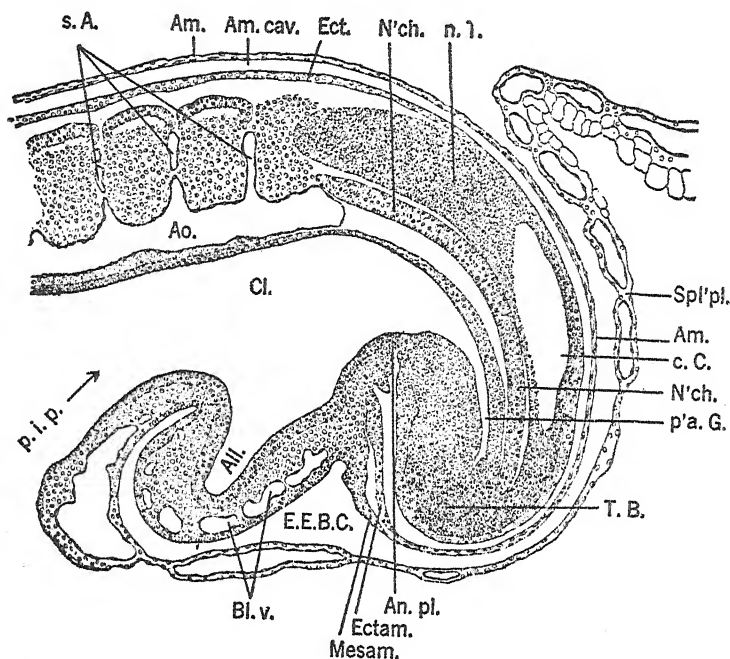


Fig. 197. — Sagittal section through the tail of an embryo of about 35 somites. From Lillie (*Development of the Chick*).

All. Allantois. Am. Amnion. Am.cav. Amniotic cavity. An.pl. Anal plate. Ao. Dorsal aorta. Bl.v. Blood-vessels in wall of allantois. c.C. Central canal of spinal cord. Cl. Cloaca. Ect. Ectoderm. Ectam. Ectoderm of amnion. E.E.B.C. Exocoelom. Mesam. Mesoderm of amnion. N'ch. Notochord. n.T. Nerve cord. p'a.G. Post-anal gut. p.i.p. Posterior intestinal portal. s.A. Segmental arteries, between the somites. Spl'pl. Splanchnopleure and yolk-sac entoderm. T.B. Tail bud.

thors, e.g., Lillie and the present writer. Gruenwald, following an old interpretation presented by Duval in his atlas, chooses to regard the original "hind-gut" as already "allantois." As can be seen from the figures, it is true that a considerable portion of the original hind-gut is eventually included in the allantoic outgrowth. It has also been shown that the elimination of this region results in more or less complete elimination of this organ (Zwilling, '46). Nevertheless, it seems to the writer confusing to identify this gut in its primary condition with the allantois,

involving as it certainly does at that time the anal plate. It seems preferable to say that the allantois grows out from the part of this hind-gut which, by the processes shown, eventually comes to lie anterior to the anal plate.

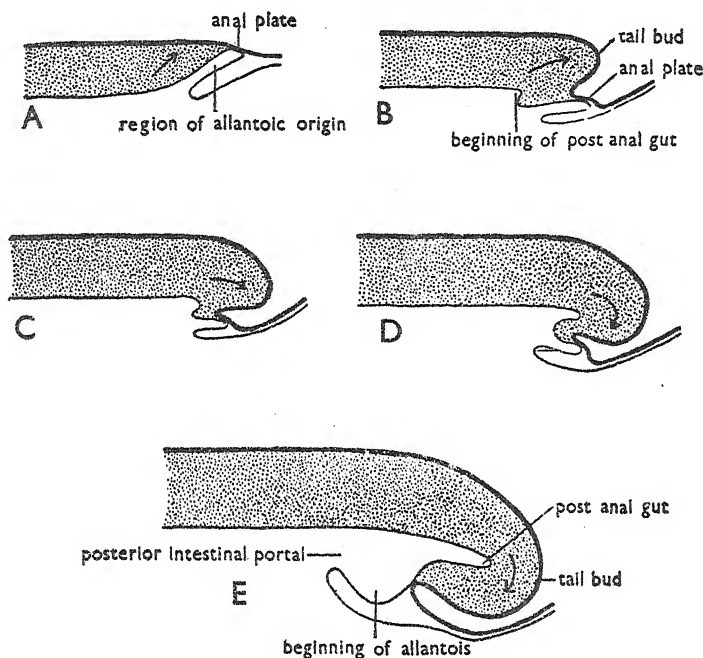


Fig. 198.—Diagrams representing changes in the tail and hind-gut region of the Chick during the third day, up to the 30 somite stage. After Gruenwald with slight modifications. The successive stages are indicated in the order of the letters.

THE CIRCULATORY SYSTEM

THE HEART

There are no very marked changes in the form of the heart during the third day, though the atrium becomes slightly more prominent, and the bendings and constrictions already described are somewhat emphasized (Fig. 199). Internally toward the end of the day sections reveal the appearance of a slight ingrowth from the atrial wall just to the left of the sinus venosus. It is the beginning of the *interatrial septum* (Quiring, '33). In the ventricular region the myocardium is becoming thick-

ened and spongy, but in the bulbus arteriosus, on the other hand, endo-thelial thickening has occurred, while the myocardium remains thin (Fig. 201).

THE EMBRYONIC BLOOD VESSELS

The Arteries.

The Dorsal Aortae. — During the third day these vessels continue their development by beginning to form posterior to the point at which the vitelline arteries leave the body.

These latter arteries thus become lateral branches of the dorsal aortae, instead of their continuations, while the further posterior growth of these aortae brings them eventually to the extremity of the tail bud. Meanwhile anteriorly they have become fused, so that by the end of the third day a single aorta extends from just back of the aortic arches almost to the origin of the vitelline arteries. Finally during the fifth and sixth days the fusion of these vessels progresses into the tail region also, resulting in the formation of a single *caudal artery*. It will not be necessary, however, to trace these processes of growth and fusion in detail.

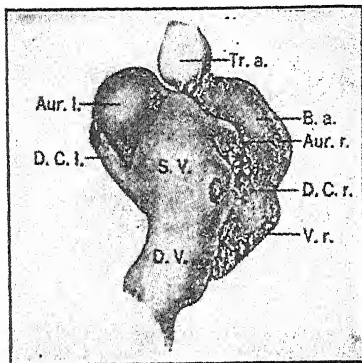


Fig. 199. — Heart of a Chick embryo of 72 hours, dissected out and drawn from the dorsal surface. From Lillie (*Development of the Chick*).

Aur. l. Left atrium. *Aur. r.* Right atrium. *B. a.* Bulbus arteriosus. *D. C. r. l.* Right and left ducts of Cuvier. *D. V.* Ductus venosus. *S. V.* Sinus venosus. *Tr. a.* Truncus arteriosus. *V. r.* Right limb of ventricle.

The Aortic Arches. — During the third day each original carotid loop plus the anterior part of each original ventral aorta disappears. At the same time the part of each original ventral aorta which occupied the ventral four-fifths of each mandibular arch becomes directly connected with its respective dorsal aorta through the upper fifth of each of these arches (Fig. 200). In this way the actual first aortic arches are completed.¹ However, before the end of the day the dorso-ventral connections of these vessels in the mandibular arches have been broken,

¹ This statement is based on figures from both Duval and Lillie. It should be pointed out, however, that Lillie does not actually say that such a direct dorsal connection occurs, and the writer has not been able to verify the point at first hand. If such a connection is established it is certainly for a very brief time, and confirmation would require the study of closely graded embryos.

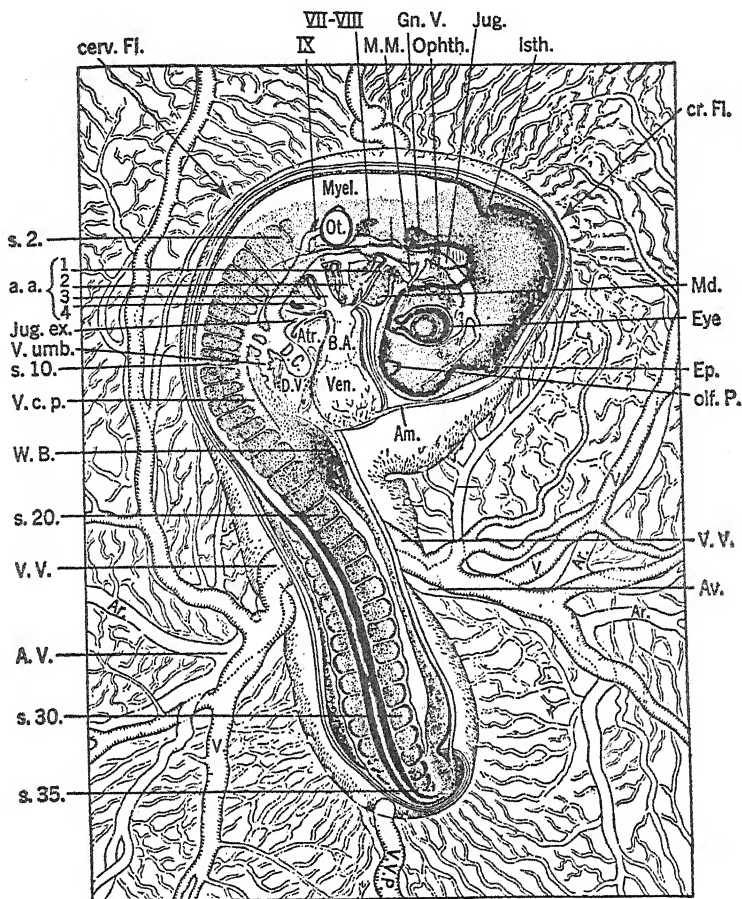


Fig. 200. — Chick embryo with adjacent portion of area vasculosa, with 35 pairs of somites (about 72 hours). Dorsal view. From Lillie (*Development of the Chick*).

a. a. 1, 2, 3, 4. First to fourth aortic arches. Am. Amnion. Ar. Branches of vitelline arteries. Atr. Atrium (Auricle). A.V. Vitelline artery. B.A. Bulbus arteriosus. cerv. Fl. Cervical flexure. cr.Fl. Cranial flexure. D.C. Ductus Cuvieri. D.V. Ductus venosus. Ep. Epiphysis. Gn.V. Ganglion of V cranial nerve. Isth. Isthmus. Jug. External jugular vein. Md. Mandibular arch. M.M. Maxillo-mandibular branch of V cranial nerve. Myel. Myelencephalon. olf.P. Olfactory pit. Ophth. Ophthalmic branch of V cranial nerve. Ot. Otocyst. s.2, s.10, s.20, etc. Second, tenth, twentieth, etc., somites. V. Branches of the vitelline veins. V.c.p. Posterior cardinal vein. V.umb. Umbilical vein. V.V. Vitelline vein. V.V.p. Posterior vein. W.B. Wing-bud.

and thus the first aortic arches vanish after a very brief existence. The dorsal aortae in this region do not disappear, however, but extend anteriorly as the *internal carotids*. Ventrally the stump of each first aortic arch persists, and presently produces an anteriorly growing twig which becomes the *primary external carotid*. (See fifth day for final development.) Meanwhile a *fourth aortic arch* arises in each of the fourth visceral arches.

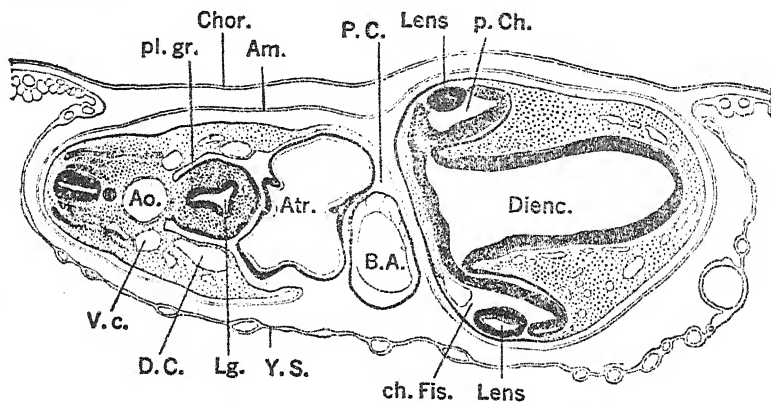


Fig. 201. — Transverse section, passing through the eyes and heart, of an embryo with about 35 pairs of somites (about 72 hours). Compare with Fig. 200. From Lillie (*Development of the Chick*).

Am. Amnion. Ao. Dorsal aorta. Atr. Atrium. B.A. Bulbus arteriosus. ch.Fis. Choroid fissure. Chor. Chorion. D.C. Ductus Cuvieri. Dienc. Diencephalon. Lg. Rudiment of lung branches. P.C. Pericardial cavity. p.Ch. Posterior (vitreous) chamber. pl.gr. Pleural groove. V.c. Posterior cardinal vein. Y.S. Yolk-sac.

The Pulmonary Arteries. — During the third day, these arteries appear as rudiments within the walls of the lungs.

The Veins.

The Cardinals and Jugulars. — During the third day, the anterior cardinals continue to branch considerably in the brain region and may now be known as the *internal jugulars*. At the same time a vessel from the floor of the pharynx joins each anterior cardinal (internal jugular) just at its point of union with the duct of Cuvier. These new veins are the *external jugulars* (Fig. 200). Late on the third day also a new pair of cardinals begins to develop. They arise from a series of anastomosing vessels on the ventral side of the mesonephros just lateral to the dorsal aorta, and are known as the *subcardinals*. They are scarcely apparent as definite vessels before the fourth day.

The Vitelline Veins. — Before leaving the body of the embryo, these veins become united by a short transverse vessel which passes over the intestine just posterior to the dorsal pancreatic rudiment. In this manner, the intestine is surrounded by a venous ring. The anterior ventral part of this ring is formed by the posterior end of the ductus venosus. The lateral parts consist of the portions of the vitelline veins lying between the ductus venosus and the transverse vessel, and the posterior dorsal part is constituted of the transverse vessel itself (Fig. 211, A, B; see Chapter 12). Meanwhile, as indicated in the account of the liver, the portion of the ductus venosus which lies within that organ is beginning to give off capillaries among the branches of the liver diverticula.

The Umbilical Veins. — Early on the third day, a vein develops in the body wall on each side of the embryo, and opens anteriorly into the respective duct of Cuvier. These are the beginnings of the *umbilical veins*, although at this

time they have no connection with the allantois (Fig. 203). Until such a connection has been established the blood from this organ is conducted to the lateral vitelline veins as follows: A transitory vessel, the *sub-intestinal vein*, develops upon the dorsal surface of the allantois, from whence it proceeds up onto the ventral side of the gut, along which it passes to the posterior intestinal portal. Here it divides into two parts which pass anteriorly around either side of the yolk-stalk to open into the vitellines as these vessels run from the yolk-sac along the margins of the anterior intestinal portal to the ductus venosus.

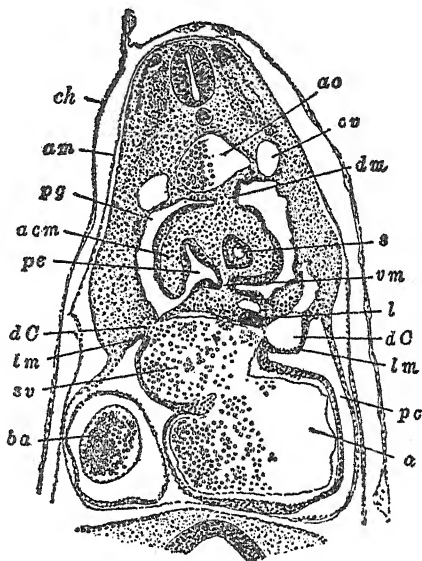


Fig. 202. — Part of a transverse section through the lateral mesocardia of a Chick with 35 pairs of somites (about 72 hours). From Kellicott (*Chordate Development*). After Lillie.

a. Atrium. acm. Accessory mesentery. am. Amnion. ao. Dorsal aorta. ba. Bulbus arteriosus. ch. Chorion. cv. Posterior cardinal vein. dC. Ductus Cuvieri. dm. Dorsal mesentery. l. Liver. lm. Lateral mesocardium. pc. Pericardial cavity. pe. Pulmoenteric recess. pg. Pleural groove. s. Stomach. sv. Sinus venosus. vm. Ventral mesentery.

THE EXTRA-EMBRYONIC BLOOD VESSELS

The Arteries.—The vitelline arteries reach further out into the area vasculosa than during the second day, terminating near its border in a network of capillaries which empty into the sinus terminalis.

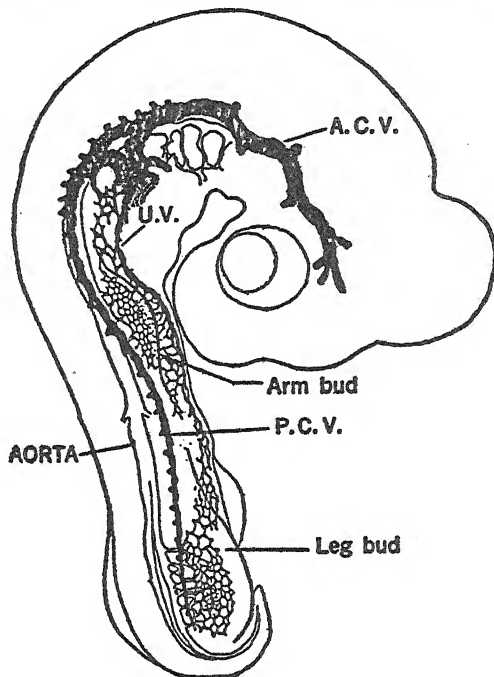


Fig. 203. — Injected Chick embryo of the third day, showing the arrangement of the cardinal veins and the formation of the umbilical vein from capillary networks. From Evans.

A.C.V. Anterior cardinal vein. *P.C.V.* Posterior cardinal vein. *U.V.* Umbilical vein.

The Veins.—Posterior to the point where the anterior vitelline veins have fused, the right vein becomes greatly reduced. During this period, also, the lateral vitelline veins passing backward and outward along the margins of the anterior intestinal portal continue to form from the vascular network lying close to either side of the embryo. In this manner, they presently reach the region where the vitelline arteries turn rather directly outward into the area vasculosa, and at this point they also begin to pass outward just dorsal to the arteries. These veins

never extend all the way to the sinus terminalis, but branch widely in the more central part of the vascular area. They receive blood from the terminalis, however, through several *intermediate veins* (venous trunks), which cross the outer network of arterial capillaries to reach them. Before the end of the third day, one other new extra-embryonic vessel starts to appear, the *posterior vitelline vein*. At this time it is scarcely more than a mass of capillaries, but very shortly begins to become distinct. It runs forward from the posterior side of the sinus terminalis, and empties into the left lateral vitelline vein near its base (Fig. 182).

THE NERVOUS SYSTEM

THE FLEXURES

These have already been discussed under the description of external changes.

THE FORE-BRAIN OR PROSENCEPHALON

The Telencephalon. — The indentation which marks the velum transversum becomes much more prominent, while the rudiments of the cerebral hemispheres grow in size and their walls increase in thickness. In about the center of the lamina terminalis, a thickening appears called the *torus transversus*. It corresponds to the similarly named structure in the Frog, and as in that case it represents the rudiment of the future *anterior commissure*.

The Diëncephalon. — The more anterior (ventral) portion of the diëncephalon is now sometimes distinguished as the *parencephalon*, and the posterior (dorsal) portion as the *synencephalon* (Fig. 204). Between them is a slight constriction, while the *parencephalon* is approximately bounded below by the marked indentation of the velum transversum. Thus the roof of the *parencephalic* region constitutes a relatively raised area from which the *epiphysis* begins to develop at the close of the day as a small out-pushing. Upon the floor of the diëncephalon, the optic recess, the region of the optic chiasma, and the infundibulum all become more pronounced than they were at the end of the second day.

THE MESENCEPHALON

The roof of the mid-brain grows rapidly and becomes prominently arched, while its walls increase uniformly in thickness. This arching of

the mid-brain causes the boundary between it and the roof of the dien-cephalon to appear gradually more constricted. Likewise posteriorly at the connection between mid- and hind-brain, a slight constriction in the roof and lateral walls, indicated during the second day, also becomes very pronounced. This latter constricted region is henceforth known as the *isthmus*.

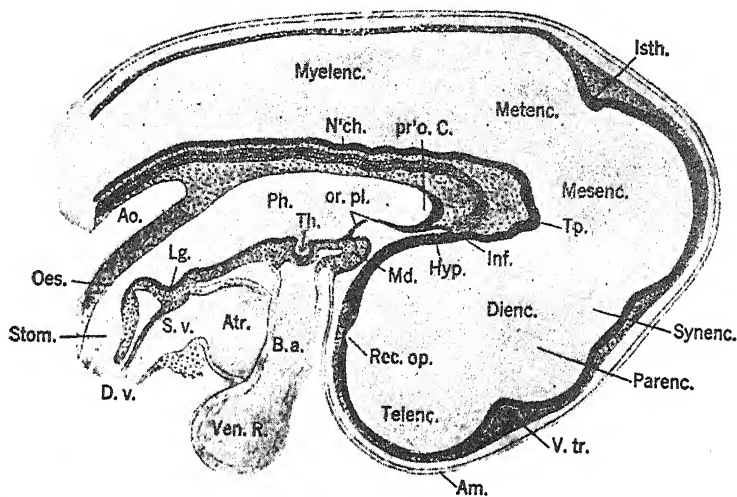


Fig. 204. — Optical longitudinal section of the head of an embryo of 30s. The heart is represented entire. From Lillie (*Development of the Chick*).

Atr. Atrium. B.a. Bulbus arteriosus. D.v. Ductus venosus. Isth. Isthmus. Lg. Laryngotracheal groove. Oes. Oesophagus. or.pl. Oral plate, which has begun to rupture. Parenc. Parencephalon. Ph. Pharynx. Stom. Stomach. Synenc. Synencephalon. Th. Thyroid. S.v. Sinus venosus. Ven.R. Right ventricle. Other abbreviations as before.

THE RHOMBENCEPHALON

The Metencephalon. — After the isthmus has become established the thickening roof of the metencephalon consists largely of the wall forming the posterior side of the constriction. By the end of the day, the lateral walls of the metencephalon have also begun to thicken.

The Myelencephalon. — The roof of the myelencephalon remains thin, while its ventro-lateral walls have started to thicken somewhat.

The Spinal Cord. — At the end of the second day, the walls of the spinal cord were seen to consist chiefly of ependymal supporting cells and germinal cells. During the third day, the latter continue to multiply, and their descendants migrate out somewhat from their position

near the central canal. In their new location, they presently become transformed either into neuroblasts, i.e., primitive nerve cells, or into primordial glia cells. The nerve cells even at this time have begun to send out the axones and dendrites typical of the adult neurones. The central parts of these neurones together with glia cells eventually come to constitute the *gray matter* of the cord, while the axones form its *white matter*.

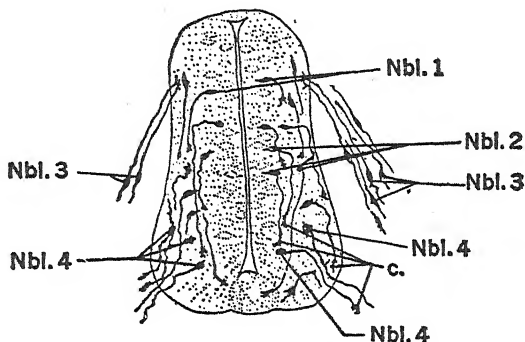


Fig. 205. — Transverse section through the spinal cord and ganglion of a Chick about the end of the third day; prepared by the method of Golgi. From Lillie (*Development of the Chick*). After Ramon y Cajal.

c. Cones of growth at the ends of growing nerve fibers. Nbl. 1 and 2. Neuroblasts of the lateral wall. Nbl. 3. Neuroblasts of the spinal ganglion. Nbl. 4. Neuroblasts of the ventral horn (motor neuroblasts).

As regards the final condition of the cord, the following may be said: Internally, the central canal is obliterated, save for a small ventral portion lined by the inner ciliated ends of the ependymal cells. Surrounding this and filling the central part of the cord is the gray matter with dorso-lateral and ventro-lateral extensions or horns reaching out into the white substance. Externally, there develops along both the dorsal and ventral sides a median longitudinal fissure. These fissures are formed mainly as a result of the enlargement of the lateral regions through the accumulation of the nerve fibers within them.

The Spinal Nerves. — The spinal nerves are sometimes described as constituting parts of two systems, (1) the *somatic*, and (2) part of the *parasympathetic* and the *sympathetic*; both systems start to develop on the third day. We shall consider the somatic system first.

I. The Somatic System. — From bipolar nerve cells within each spinal ganglion one bundle of fibers (dorsal root) grows into the spinal

cord, and another outward in a ventro-lateral direction. Together these constitute the *afferent* or sensory nerve fibers. At the same time from the ventro-lateral side of the nerve cord beneath each spinal ganglion, fibers (ventral root) are growing out from nerve cells located within the cord. These are *efferent* or *motor fibers* which mingle with those of the respective outgrowing afferent bundle just at the point where the latter leave their ganglion. The mixed fibers thus form the *common trunk* of a somatic spinal nerve. This trunk then divides again into a dorsal and ventral part, each part containing fibers of both the above types. The condition thus indicated is approximately the stage reached in the development of the somatic nerves at the end of the third day or early on the fourth (Fig. 205; common trunk not shown).

Inasmuch as it will not be profitable in a work of this scope to follow further the detailed development of the somatic spinal nerves from day to day, their future arrangement will be summed up at this time, as follows: The fibers of the divided trunks increase in number and at the same time grow outward. Hence, they almost immediately come into contact with the muscular and dermal plates, which are the rudiments of the future voluntary musculature and dermis of the Chick. Thus nervous connections are early established with these elements, and as the latter develop, the nerves (motor and sensory) develop with them.

It should be noted that some of this musculature just indicated is destined for the limbs, and hence certain groups of the spinal nerves will constitute the brachial and the sciatic plexuses. In this connection certain experimental results are of interest. Thus it has been shown that when limb buds are transplanted to abnormal locations as described above, spinal nerves nearby, which would normally have nothing to do with limbs, are apparently "attracted to them," even forming a characteristic plexus before entering them (Hamburger, '39). (However, see conclusions of Detwiler and Piatt on this matter in the section on the Frog). Hamburger ('39, '44, '49), Bueker ('45) and others have also shown that the number of motor neurons in the cord may be respectively decreased or increased by the extirpation of an adjacent limb bud or the implantation of an extra one. Hamburger also showed that the variation in number of motor neurons was apparently not caused by a difference in the total number of cells, but rather by the differentiation of more or less of this particular type of cell as compared with other types. These results show the effect of developing limb buds on nerves. Lastly, however, Hunt ('32) and Eastlick ('43) have demonstrated that in trans-

planted limbs which for any reason fail to be innervated few muscle fibers develop, and those that do, degenerate after about ten days. In conclusion it thus appears that there are reciprocal influences between a growing limb bud and its musculature on the one hand, and the development of neurons and their fibers on the other.

II. The Sympathetic and Sacral Parasympathetic Systems. — As in the Frog there has been much disagreement concerning certain details of the origin of parts of these systems. For some time all postganglionic neurons at least were alleged to arise from neuroblasts in the dorsal root ganglia, i.e., originally from the neural crests. Later Jones, '37, '39, '41 asserted that cells within the neural tube were the exclusive source for these systems. Further experimental study by Hammond, '49 and Yntema and Hammond, '54 '55 seem now to have resolved the problem as follows: It appears that all postganglionic neurons and their fibers are derived from the neural crest. All preganglionic fibers, both sacral parasympathetic and thoraco-lumbar sympathetic arise from special aggregations of motor neurons within the spinal cord. The sheath cells of all the fibers are from the crest and tube (Brizzee, '49), and possibly some mesoderm.

At the end of the third day or early on the fourth the postganglionic cells derived from the crest collect just above and to either side of the dorsal aorta. Here they send out fibers anteriorly and posteriorly, forming a pair of delicate longitudinal cords running from the cervical region to the tail, with thickenings (ganglia) opposite each somatic ganglion. These are the *primary sympathetic* and *sacral parasympathetic cords* and *ganglia*, and each of these ganglia is connected with a somatic ganglion by a strand of fibers, the *primary rami communicantes*. Lastly there are a few cells in the dorsal mesentery, probably from the crest, and destined to form Remak's ganglion (Chap. 12, Fig. 216).

The Cranial Ganglia and Nerves. — The ganglia of the V, VII, VIII, IX and X nerves have already been described as appearing on the second day. During the third day, the V ganglion shifts its position of attachment to the brain somewhat, and its characteristic Y shape becomes more marked. The VII and VIII ganglionic mass also shifts to a more dorsal position. Otherwise the cranial ganglia show no marked alterations at this time (Fig. 200).

The Mixed Character of Certain Cranial Nerves. — In the Chick, as in the Frog, it is possible to distinguish the V, VII, IX and X nerves as mixed, i.e., as containing both sensory and motor elements. In this respect they are of course not different from the spinal nerves, except as

regards the point at which the two types of fibers become mingled. Thus in the region of the cord, the ventral or motor fibers of any nerve join the dorsal or sensory fibers of that nerve slightly *peripheral* to the dorsal ganglion. In the mixed cranial nerves, on the other hand, the two types of fibers issue from the brain very close together and mingle before entering the ganglion of the respective nerve. It may be further noted that though the ganglion of the VIII nerve is very closely associated at this time with that of the VII, its fibers are wholly sensory.

The III or Oculo-Motor Nerve. — Besides the mixed or wholly sensory nerves in the Chick, there are also, as in the Frog, certain cranial nerves which are purely motor and without any connection with the cranial ganglia. They take their origin from neuroblasts within the brain itself, just as spinal motor fibers arise from neuroblasts within the spinal cord. The III or oculo-motor nerve arises in this manner from the median line of the ventral side of the mid-brain, at about sixty hours. Its history will be traced a few steps further in connection with the IV and VI nerves which arise on subsequent days.

THE ORGANS OF SPECIAL SENSE

THE EYE

The Optic Cup. — There are two main changes connected with the optic cup during the third day. The first change is the rapid increase in its size. Thus at the end of the second day the lens rudiment practically filled the cavity of the cup, and came in contact with its inner wall. At the end of seventy-two hours, on the other hand, the lens is entirely separated from the wall of the cup, and simply rests within its rim. The second change is the thickening of the inner wall, from whose neuroblasts axones start to grow at the 30-somite stage (courtesy Rogers, K. T.). The optic stalk is still ventral at the point of attachment to the cup, the region surrounding this point being called the *fundus* (Fig. 201).

The Lens. — The lens becomes detached from the superficial ectoderm during the third day, and forms a hollow ball, whose walls are at first of almost uniform thickness. Presently, however, the cells of the inner wall (i.e., the one next to the optic cup) begin to lengthen, in a direction at right angles to this wall, so that the latter is thereby thickened. By the end of the day this thickening has progressed to a considerable extent, the elongated cells which cause it being destined to form the lens fibers, which constitute the core of the lens.

THE EAR

At the end of the second day, the auditory pit had been transformed into the auditory sac, whose mouth was still partly open to the exterior. By virtue of the method of the closure of the pit, described in the previous chapter, the major part of the sac lies below the level of its external

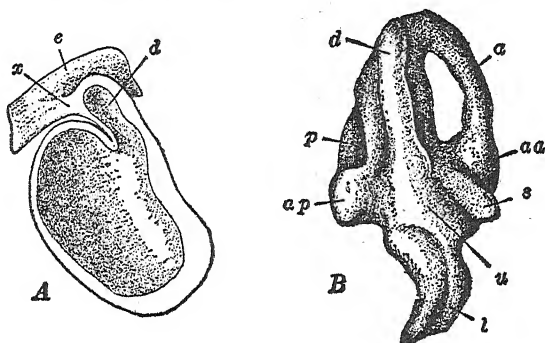


Fig. 206.—Two stages in the development of the auditory organ of the Chick. From Kellicott (*Chordate Development*. A. Hemisected model of left auditory sac posterior view, just before the separation from the head ectoderm, at about 72 hours. After Krause. B. Median view of a model of the left membranous labyrinth of an embryo of 7 days and 17 hours. After Röthig and Brugsch.

a. Anterior vertical semicircular canal. aa. Ampulla of anterior vertical semicircular canal. ap. Ampulla of posterior vertical semicircular canal. d. Ductus endolymphaticus. e. Superficial ectoderm of head. l. Lagena (cochlea). p. Rudiment of posterior vertical semicircular canal. s. Rudiment of saccule. u. Utricle. x. Connection between auditory sac and superficial ectoderm.

orifice. The connection of this orifice with the dorsal portion of the sac is then drawn out into a narrow tube, while the dorsal part of the sac itself is at the same time slightly constricted away from the major ventral part. The former, or dorsal portion, is the rudiment of the *endolymphatic duct*, which presently grows upward somewhat so that its roof is slightly dorsal to the level at which the tube leading from it opens to the exterior (Fig. 206, A).

THE OLFACTORY ORGANS

Early on the third day a small circular spot of ectoderm on each ventro-lateral side of the head somewhat in front of the eye becomes thickened, in consequence of a lengthening of its cells. These patches

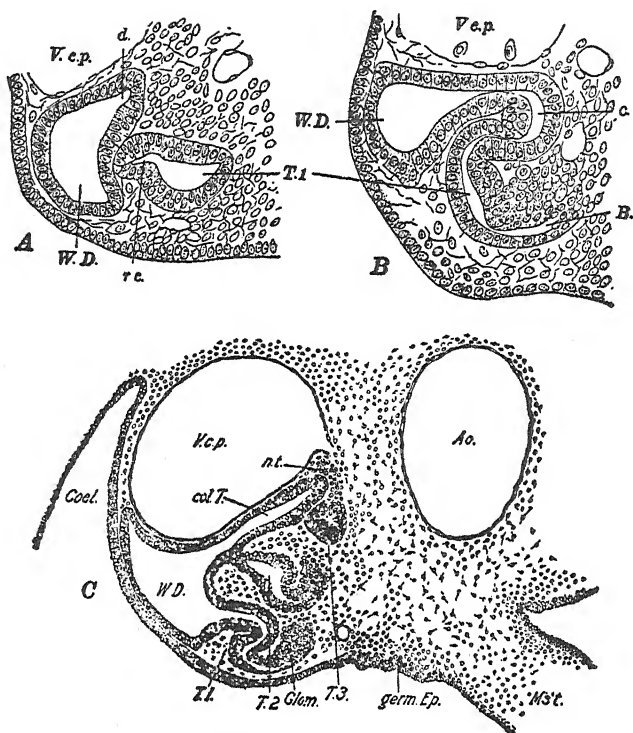


Fig. 207.—The development of the mesonephros. *A.B.* Transverse sections through the mesonephric tubules of the Duck embryo with 45 pairs of somites. From Kellicott (*Chordate Development*). After Schreiner. *C.* Transverse section through the middle of the mesonephros of a Chick of 96 hours. From Lillie (*Development of the Chick*).

Ao. Dorsal aorta. *B.* Rudiment of Bowman's capsule. *c.* Conducting part of a primary tubule. *coel.* Coelom. *col.T.* Collecting tubule. *d.* Dorsal outgrowth of the Wolffian duct to form a collecting tubule (see fourth day). *Glom.* Glomerulus. *germ.Ep.* Germinal epithelium. *M'st.* Mesentery. *nt.* Nephrogenous tissue. *rc.* Rudiment of conducting portion of primary tubule. *T. 1, 2, 3.* Primary, secondary, and tertiary mesonephric tubules. *V.c.p.* Posterior cardinal vein. *W.D.* Wolffian duct.

then begin to invaginate, and thus form the *olfactory pits* (Fig. 200). The thickened epithelium which lines them is the *olfactory epithelium*, and is said to consist of two types of cells, simple epithelial cells and germinal cells. The latter type later give rise to neuroblasts which eventually produce the sensory cells of the olfactory epithelium, while they in turn give rise to axones which constitute the olfactory nerve. (See next chapter.)

THE URINOGENITAL SYSTEM

During the third day, the pronephros degenerates, while the mesonephros continues to develop, and soon becomes the primary excretory organ during embryonic life in a manner about to be indicated. Neither the metanephros nor the reproductive system appears during the third day.

As regards the changes in the mesonephric region, it will be recalled that at the end of the second day the Wolffian or mesonephric portion of the pronephric duct was just beginning to acquire a lumen. Its backward-growing end, however, was still solid, and had not yet reached the cloaca. On the third day, this cellular rod connects with the cloaca, and by the end of the day a lumen has formed throughout its length. Concerning the mesonephros proper, at 48 hours the rudiments of the mesonephric tubules were forming in the neighborhood of the twentieth somite or segment, i.e., in the most anterior region of the future organ. At that time, these rudiments, of which there were two or more to the somite, consisted merely of spherical condensations of the nephrotome, which were beginning to become vesicular. Now at the end of seventy-two hours, however, the vesicles opposite the most anterior mesonephric somites are giving rise to small, hollow evaginations in the direction of the Wolffian duct (Fig. 207, A). There is one evagination to each vesicle, and it is the part of the vesicle which is destined to form the actual mesonephric tubule. Indeed, just anterior to the twentieth somite or mesonephric region proper, some of the out-pushings have already become tubules and are connected through conducting portions with the Wolffian duct (Fig. 207, B). In this region also Malpighian bodies have appeared in connection with some of the tubules. These most anterior tubules and glomeruli, however, never become functional.

SUMMARY OF THE CONDITION AT THE END OF THE THIRD DAY OF INCUBATION

I. GENERAL APPEARANCE

The *cranial* and *cervical flexures* have increased, especially the latter. A small *caudal flexure* has appeared, and the region in between has developed a slight ventral curvature. The *lateral rotation* has progressed so that the embryo is on its side as far back as the twenty-first somite. The four *limb buds* are clearly visible.

II. THE SOMITES

The number of pairs of somites has increased to thirty-six and in the more anterior pairs *dermatomes* and *myotomes* are completely developed. *Sclerotomal tissue* is still collecting about the notochord and the sides of the nerve cord.

III. THE ALIMENTARY TRACT

The Fore-gut. — The *oral plate* has broken through to complete the *oral cavity*, and Rathke's pocket reaches nearly to the *infundibulum*. Subsequent development of these parts to form the *pituitary* is described in this chapter. The *second pair of visceral pouches* has acquired clefts, and the *fourth pair* has fused with the ectoderm. The *thyroid* depression has become a sac. The depression indicating the *respiratory system* has deepened in the *laryngotracheal groove*, and the *rudiments of the lungs* have appeared. The *esophagus* and *stomach* are beginning to be defined. Finally, the *liver diverticula* have grown forward and anastomosed about the posterior part of the ductus venosus; the rudiment of the *gall bladder* is visible, and the dorsal portion of the *pancreas* has appeared.

The Mid-gut. — It has become more clearly defined.

The Hind-gut. — The *anal plate* has been carried around to the ventral side by the growth of the tail bud, and at the same time the *postanal gut* has been formed. The rudiment of the *allantois* has appeared.

IV. THE CIRCULATORY SYSTEM

The Heart. — There are no external changes aside from an emphasis of curvatures and constrictions already present. In the *ventricular region* myocardial thickening has occurred, and in the *bulbus arteriosus* the same is true of the endothelium. The *interatrial septum* has started to form.

Embryonic Arteries. — Fusion of the *aortae* has progressed. The *first pair of aortic arches* has been completed and then disappeared. The dorsal aortae extend anteriorly as the *internal carotids*, while the stumps of the first arches produce the *external carotids*. The *fourth pair of arches* has developed, and the rudiments of the *pulmonary arteries* have arisen in the lungs.

Embryonic Veins. — The *anterior cardinals* have branched considerably in the brain region and are now known as the *internal jugulars*

which receive the *external jugulars* just at the union of the former with the ducts of Cuvier. The *ductus venosus* is beginning to develop capillaries among the branching liver diverticula. A new vessel passes over the intestine in the neighborhood of the pancreas and unites the vitelline veins to form a ring about the alimentary tract. A longitudinal vein has developed in each body wall; they are the *umbilical veins*, though at this time neither has acquired a connection with the allantois. The rudiments of the *subcardinal* veins may be visible on the ventral side of the mesonephros. The transitory *subintestinal* vein is present.

Extra-embryonic Arteries.—The *vitelline arteries* have pushed out into the area vasculosa until their branches nearly reach the sinus terminalis.

Extra-embryonic Veins.—The *right anterior vitelline* vein has almost disappeared; the *posterior* and *intermediate* vitelline veins have started to arise, and the *lateral vitelline* veins have developed further.

V. THE NERVOUS SYSTEM

The Flexures and the Brain.—As noted under external appearance the *cranial* and *cervical flexures* are both increased. The *cerebral hemispheres* have grown somewhat, and the *epiphysis* has started to develop. The *optic chiasma*, the *optic recess*, and the *infundibulum* have all become more clearly marked. The roof of the *mid-brain* is more prominently arched and the *isthmus* has appeared. There has also been thickening and thinning of the brain walls at various points.

The Spinal Cord and Spinal Nerves.—The *germinal cells* have changed their position and have begun to develop into *neurones* and *glia* cells. The *sensory* and *motor* nerve fibers issue respectively from the spinal ganglia and the ventral portion of the cord, the two types uniting to form the *common trunks* of the *somatic spinal nerves*. The *primary sympathetic trunks*, *ganglia* and *communicating rami* have appeared. The completion of the somatic portion of the spinal nervous system is described in this chapter.

The Cranial Ganglia and Nerves.—The ganglia have shifted their position slightly, and the third or *oculo-motor* nerves have appeared.

VI. ORGANS OF SPECIAL SENSE

The Eye.—The *optic cup* has increased in size and its inner wall has thickened. The *lens* has become detached from the ectoderm, and its inner wall is also thickening.

The Ear. — The rudiment of the *endolymphatic* duct has appeared on the dorsal portion of the auditory sac.

The Olfactory Organs. — The *olfactory pits* have been formed, with walls consisting of epithelial and germinal cells.

VII. THE URINOGENITAL SYSTEM

The *pronephros* has begun to degenerate, while the *mesonephros* has started to develop tubules and glomeruli in its most anterior portion. The *Wolffian duct* has reached the cloaca and acquired a lumen throughout its length.

VIII. THE AMNION AND ALLANTOIS

The folds of the *amnion* have approached one another above the posterior portion of the embryo and formed the *amniotic umbilicus*. The *allantois*, by about the middle of the day, has the appearance of a slight out-pushing from the hind-gut, and by the close of the day has extended well into the somatic umbilicus.

T

THE CHICK: DEVELOPMENT DURING THE FOURTH DAY OF INCUBATION

GENERAL APPEARANCE

FLEXURES AND TORSION

THE *cranial flexure* remains about as on the previous day, but the *cervical flexure* has increased so in degree and extent as to bring the whole head further posterior. Also it brings the region of the *diencephalon* around so that it and the anterior part of the optic vesicles face almost directly caudad. At the same time the mid-region of the cervical flexure is now the most anterior part of the embryo. From the anterior to the posterior limb buds the longitudinal axis has in most cases lost its ventral curvature, and has become virtually straight. Caudad to the posterior limb bud the *caudal flexure* is more marked so that the tip of the tail is curled around beneath the body. The *lateral torsion* now extends throughout the whole embryo so that it lies entirely on its side.

THE LIMB BUDS

All the limb buds have increased in prominence.

THE SOMITES

THE COMPLETION OF THEIR FORMATION

By the end of the fourth day the number of somites has reached 42, and subsequent to this time ten more are added posteriorly. These last ten, however, later disappear, together with the four most anterior ones (head somites), which become fused with the skull. Thus at 96 hours the Chick possesses all the somites which take any part in the development of the adult Bird. The development of the myotomal and dermatomal elements progresses posteriorly in the manner already described.

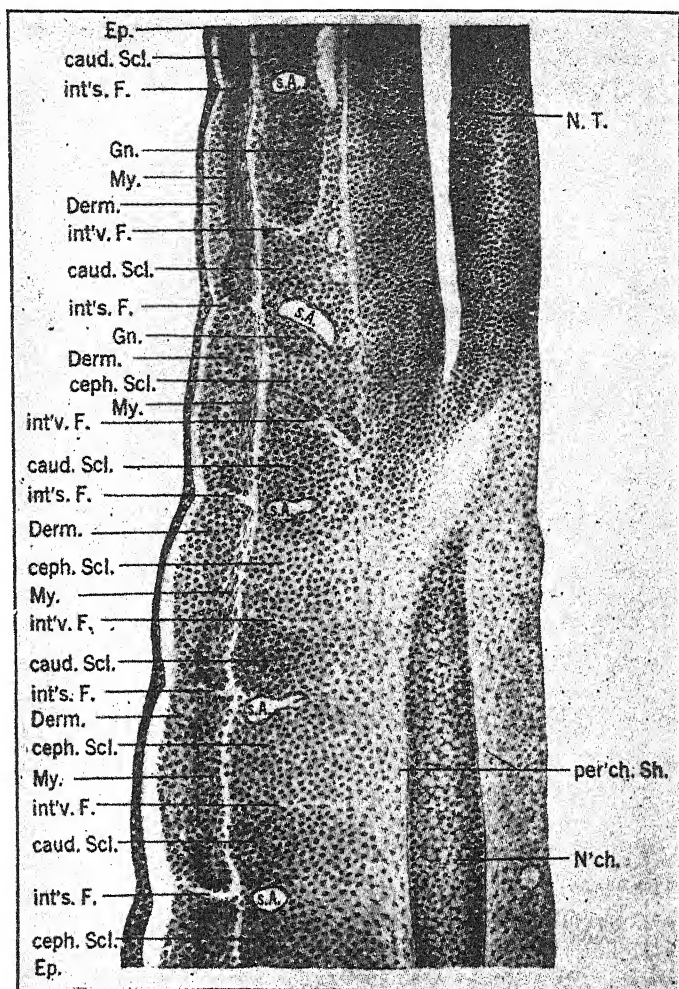


Fig. 208. — Frontal section through the base of the tail of a Chick embryo of 96 hours. The anterior end of the section (above in the figure) is at a higher plane than the posterior end. From Lillie (*Development of the Chick*).

caud.Scl. Caudal division of the sclerotome. *ceph.Scl.* Cephalic division of the sclerotome. *Derm.* Dermatome. *Ep.* Epidermis. *Gn.* Ganglion. *int's.F.* Intersomitic fissure. *int'v.F.* Intervertebral fissure. *My.* Myotome. *N'ch.* Notochord. *N.T.* Neural tube. *per'ch.Sh.* Perichordal sheath. *s.a.* Segmental artery.

THE ULTIMATE FATE OF MYOTOMES AND DERMATOMES

Although the ultimate disposition of these elements of the somites is not accomplished until some time later, it is not desirable to follow their development longer by one-day periods. Regarding the dermatomes, or cutis plates, it has already been stated that their substance gradually moves out beneath the ectoderm, and ultimately forms the dermis in the dorsal regions, the dermis in the more ventral parts being derived from the underlying somatopleure. Likewise Straus and Rawles, '53 have now shown by carbon marking that the myotomes also are the source of only about the upper one third of the voluntary body muscles plus parts of three in the abdomen, the rest being somatopleural in origin. Head musculature and involuntary muscles develop from mesenchyme.

THE SCLEROTOMES

During the third and fourth days the mesenchyme of the sclerotomes comes to occupy all spaces about the notochord and between the latter and the myotomes. Indeed, immediately around the notochord itself it forms a thin continuous layer, the *perichordal sheath*. Further peripherally, however, a concentration of the mesenchyme in the cephalic and caudal portion of each sclerotome, as well as a slight division between these portions, has long made these parts distinguishable as such. Upon the fourth day, moreover, it begins to appear that upon either side of the notochord the cephalic half of each sclerotome is beginning to become fused with the caudal half of the one anterior to it, thereby establishing a new segmental arrangement (Fig. 208). From the method of their formation, it follows that the segments thus arising do not coincide with the myotomes; instead, they alternate with them just as they did in the Frog. In this manner, blocks of mesenchyme are being marked out on either side of the notochord; these are the rudiments of the right and left halves of the future *vertebrae*. Lastly, from the cephalic and caudal portion of each sclerotome, mesenchymatous tissue has now extended well upward around the sides of the nerve cord. This forms the rudiments of the *neural arches*, the cephalic arch of one sclerotome later fusing with the caudal of the next to form single arches corresponding to the *vertebrae*. The reason for the development of the alternative arrangement between *vertebrae* and myotomes, i.e., muscles, should be quite evident. In order to bend the back or neck it is apparent that each set of muscles must be attached at each of its ends to a different vertebra.

THE ALIMENTARY TRACT

THE REGION OF THE FORE-GUT

The Tongue. — The tongue appears on the fourth day as two papilliform outgrowths from the floor of the pharynx, one in front of and one behind the thyroid. These two rudiments then grow forward and fuse with one another. Eventually the structure thus constituted unites with a pair of lateral folds to form the tongue of the adult.

The Visceral Pouches and Arches.

The Pouches. — During the fourth day, the third pair of pouches acquire dorsal and ventral clefts like those of the second, while the clefts of the latter pouches and of the first (hyomandibulars) become closed. The second pouches then gradually disappear, whereas the dorsal portions of the first pair extend dorso-posteriorly toward the respective otocysts; here each eventually forms a part of the tubo-tympanic cavity (see fifth day).

The Arches. — The five pairs of arches reach their maximum development as such during the fourth day, and certain changes in their blood vessels take place; these changes will be described below.

The Thyroid. — The thyroid sac at this time completely separates from the floor of the pharynx. Subsequently it becomes divided into two massive lobes which move backward and take up a position at the junction of the subclavian and the common carotid arteries. The effect of the pituitary upon the later development of this gland has been determined experimentally as follows:

Transplants have been made of thyroid glands from twelve-day old Chicks to the chorio-allantoic membranes of Chicks with and without pituitaries. It was found that only in Chicks possessing the pituitary does either a transplanted thyroid or that of the host develop beyond the twelve-day stage (Martindale, '41).

The Respiratory Tract. — It will be recalled that at the end of the third day, the posterior part of the pharynx had deepened and narrowed to form the laryngotracheal groove, with the lung primordia at its posterior extremity. During the fourth day, the posterior portion of this groove, including the lung diverticula, separates from the ventral part of the alimentary tract. The anterior portion of the new tube thus formed is the *larynx* which continues to open into the pharynx through a slit-like aperture, the *glottis*. The remainder of the tube is the *trachea*,

FOURTH DAY: THE REGION OF THE FORE-GUT 399

which divides into the lung primordia, really only the primary bronchi, at its posterior end. This is the condition of the respiratory apparatus at the end of 96 hours.

The Esophagus, the Stomach, and the Duodenum. — At the end of the third day, the fore-gut region posterior to the pharynx consisted of an elongated tube — the esophagus, a slight dilation — the stomach, and finally another elongated region to which were attached the rudiments of the liver and pancreas. This last section of the fore-gut may from now on be termed the *duodenum*. During the fourth day the elongation of these parts continues, and also a certain curvature becomes evident. This latter process extends from the posterior region of the esophagus to the end of the duodenum, and the direction of the bending is such that the convex side of the curve is toward the left.

The Liver. — It will be recalled that at the end of the third day the main body of this organ had formed an anastomosing network about the ductus venosus, and that it extended somewhat further forward on the left side than on the right. During the fourth day, this network increases, together with its interstitial blood vessels (Fig. 196, *B*). As this enlargement proceeds, it will be found that the larger part of the organ comes to lie more and more upon the right side of the body, in the hollow made by the bend of the stomach.

The Pancreas. — At the close of the third day, a thickening in the dorsal wall of the intestine opposite the posterior liver diverticulum was noted as the first rudiment of the pancreas. Upon the fourth day this thickening becomes a solid outgrowth, somewhat hollowed at its base. By the end of the day, two similar ventral rudiments may also be visible as antero-lateral outgrowths from the common bile duct (the ductus choledochus). The subsequent union of these three elements will be described in the following chapter.

The Spleen. — Although this organ is not really a part of the digestive tract at all, it is convenient to describe its development at this point. During the fourth day a proliferation of cells occurs in the peritoneum at the base of the dorsal mesentery just above the dorsal pancreatic element. These cells become mingled with the surrounding mesenchymal tissue, thus forming the main substance of the *spleen*. Subsequent development results in the formation of a considerable mass, filled with sinuses which communicate directly with the splenic veins. Cells from the spleen are budded off into these spaces and pass into the circulation, where they apparently become transformed into blood corpuscles.

THE REGION OF THE MID-GUT

For purposes of definition, the fore-gut region may be said to terminate at the end of the duodenum, and this point is marked approximately by the opening of the bile duct. The mid-gut, therefore, is the portion of the alimentary tract extending from the opening of this duct to the point at which the gut contained in the tail fold begins. It is difficult to define the latter point exactly at this time, except to say that since the tail fold never becomes very deep, it is relatively near the posterior end of the embryo, a short distance in front of the origin of the allantois. This boundary between the mid- and hind-gut is marked later by the intestinal caeca (see Chapter 13).

During the third and fourth days the folding-in process has been going on rapidly in the region of the mid-gut, and due to this, and to the growth of the entire body, the somatic umbilicus is so relatively constricted as to be called the *umbilical stalk*. Within it, as already noted, are the allantoic stalk and the yolk-stalk. The former has always been small, and the latter has necessarily shared in the constriction of the umbilical walls. The result of these processes is obviously a mid-gut closed in at every point save the relatively narrow opening into the yolk-stalk; it is also a gut which still remains virtually straight. The section of alimentary tract which has thus been defined is destined to become the *small intestine* of the adult bird.

In concluding the discussion of this topic it is well for the student to realize that there are two aspects to the umbilical constrictions just indicated. There is, on the one hand, the absolute narrowing of the umbilical opening. There is also in addition to this the immense growth of the remainder of the embryo. The girth of the umbilicus is thus a relative as well as an absolute matter, and the apparent reduction in its size is due as much or more to the increase in size of the embryo as to its own constriction.

THE REGION OF THE HIND-GUT

The remainder of the digestive tract posterior to the small intestine is, by the above definition, the hind-gut, and constitutes the *large intestine* or *rectum*. This opens into a terminal chamber, the *cloaca*. There is little to be said about the development of the rectum at this time, since it remains short, uncoiled, and without appendages.

The cloaca at 96 hours consists of a chamber into whose antero-dorsal wall there opens, as indicated, the rectum. Just back of the rectal

orifice, the cloacal cavity also receives the Wolffian ducts. Antero-ventrally below the rectal opening is the aperture of the allantois, while just behind this on the ventral side of the chamber is the original anal plate, or cloacal membrane (Fig. 193). It consists, as will be recalled, of a fused plate of endoderm and ectoderm, and during embryonic life separates the cavity of the cloaca from the exterior. Posterior to these apertures and the cloacal membrane, the cloacal chamber shows a marked lateral compression.

THE CIRCULATORY SYSTEM

THE HEART

In order to understand the development of the heart during the fourth and subsequent days, it will be necessary for the reader to refer to the description of that organ at the end of the second day. Assuming that this description is clearly in mind, we may then continue the account of the development on the fourth day, as follows:

Changes in the Proportion of the Parts.—The entire loop has gradually been expanding so that its parts have tended to approach one another. This has also resulted in a relative shortening of the two ascending limbs, i.e., the posterior limb comprising the atrium and part of the ventricle, and the anterior limb comprising another part of the ventricle and the bulbus arteriosus. At the same time so great has been the expansion of the transverse portion of the loop connecting these two limbs that the limbs as such have almost disappeared. What remains of the posterior one is marked by what amounts to a constriction just below the developing atrium. This apparent constriction, however, is brought about not so much by an actual contracting of this region as by the relative expansion of the parts above and below it. Since the part above forms the atrium, and the part below is a portion of the ventricle, the constriction between constitutes the *atrio-ventricular canal*.

Changes in the Relative Position of the Parts.—At the same time that these changes in shape and proportion have been occurring, changes in the relative positions of the parts are also progressing. Of these there are three principal ones which may be indicated thus: (1) The bulbus arteriosus is swinging toward the median line beneath the atrium (Fig. 209, *D*). (2) The ventricular region is moving backward behind the atrium and also somewhat toward the median line, the region of the future apex pointing posteriorly. (3) To some degree as

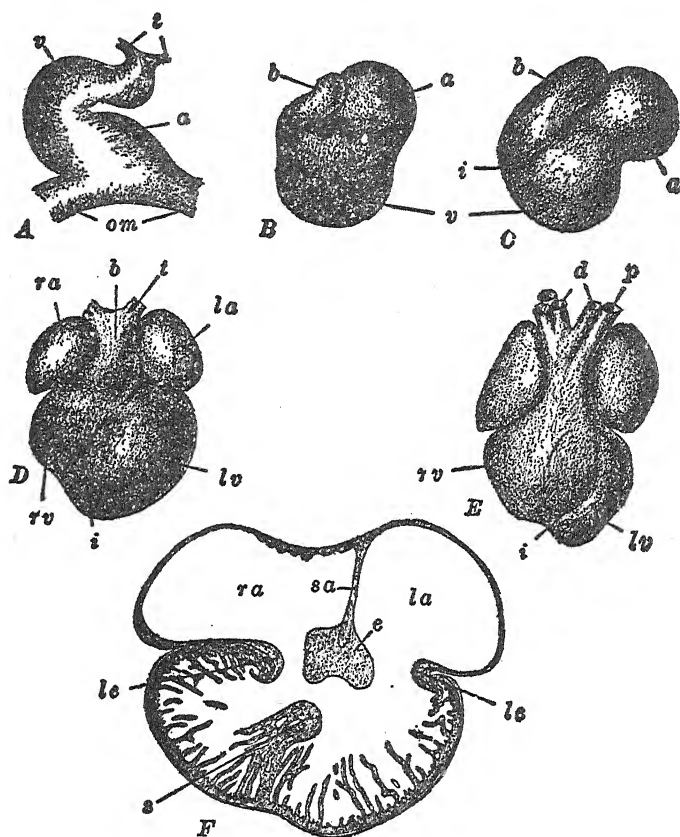


Fig. 209. — The development of the heart of the Chick. From Kellicott (*Chordate Development*). A, F, after Hochstetter. B-E, after Greil. A-E, ventral views of the heart. A. of a 40-hour embryo; B. of an embryo of 2.1 mm. head-length; C. of an embryo of 3.0 mm. head-length; D. of an embryo of 5.0 mm. head-length; E. of an embryo of 6.5 mm. head-length. F. Frontal section through the heart of an embryo of 9 mm. head-length.

a. Atrium. b. Bulbus. d. Roots of dorsal aorta. e. Median endothelial cushion (i.e., the cushion septum). i. Interventricular groove. la. Left atrium. lc. Lateral endothelial cushion. lv. Left ventricle. om. Vitelline veins. p. Left pulmonary artery. ra. Right atrium. rv. Right ventricle. s. Interventricular septum. sa. Interatrial septum. t. Roots of aortic arches. Ventricle.

a part of the latter movement, the posterior portion of the atrium into which the sinus venosus opens is rotating forward. In this manner, it is brought just over and then anterior to the atrio-ventricular canal, the latter remaining at a comparatively fixed point between the ventricular and atrial regions. Though not completed during the fourth day, these movements are well under way at this time. Their progress, moreover, is sufficient to show that their tendency is to place the parts of the heart more nearly in their adult positions; i.e., the atrium anterior and dorsal, and the ventricle posterior and ventral.

Interior Changes Involving the Growth of Septa.— While the above external alterations in the form of the heart have been going on, further internal changes are occurring as follows: (1) the interatrial septum which started to form on the third day becomes more clearly evident as a sickle shaped membrane extending postero-ventrally from the curved antero-dorsal wall, the back of the sickle being attached to the wall. Eventually of course this septum, augmented by certain other elements, completely divides the atrium into right and left chambers (the atria). (2) At the apex of the ventricle, the *interventricular septum* arises, and grows forward. Now since the ventricular apex has become posterior to both the atrio-ventricular canal and the bulbus arteriosus, it is possible for the forward extension of this septum to meet them both. This, it eventually does (see Chapter 13). (3) At the same time these septa are developing, a third one is beginning to arise within the atrio-ventricular canal; it starts as two endothelial thickenings, one in the floor, and the other in the roof of this canal. These are destined to grow towards one another until they unite in the center of the atrio-ventricular aperture, thus dividing it into right and left parts. When completed, this partition is known as the *cushion septum* (Fig. 209, F).

EMBRYONIC BLOOD VESSELS

The Arteries.

The Aortic Arches.— It will be recalled that during the third day, the first pair of aortic arches disappeared, leaving the anterior extensions of the dorsal aortae as the internal carotids. In a similar manner, extensions from the bases of the first arches continue anteriorly as the external carotids. Upon the fourth day, the second aortic arches are likewise obliterated, and the two pairs of carotids continue posteriorly to the dorsal and ventral ends of the third pair of arches. At the same time two new pairs of aortic blood vessels develop in the vestigial fifth visceral arches behind the fourth and last pair of visceral pouches.

These are the *fifth* and *sixth aortic arches* (Fig. 210, *A*). The fifth pair is small and quite transitory, being actually attached both dorsally and ventrally to the anterior sides of the sixth pair. Shortly after the sixth arches have thus arisen a small branch develops from about the middle of each and connects with the rudiments of the pulmonary arteries growing out from the lungs. In this manner the pulmonary arterial system is completed, though throughout embryonic life the branches just indicated remain small.

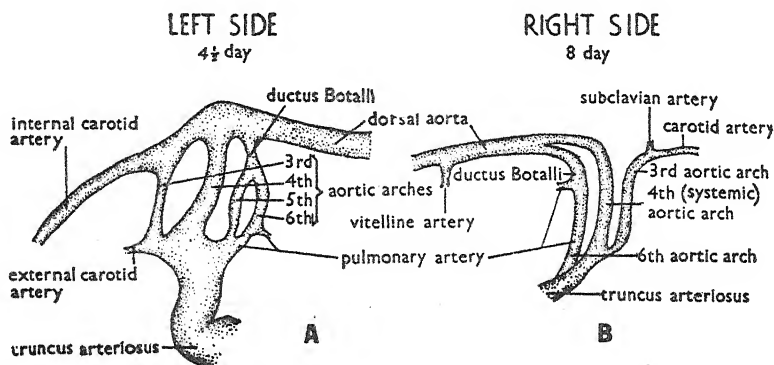


Fig. 210. — Aortic arches of the Chick. Left side from a 4½-day injected embryo. Modified from Lillie, after Locy. Right side reconstructed from sagittal sections of an 8-day embryo. Modified from Lillie.

From this description, it is clear that only the ventral portions of the sixth arches take part in the formation of the pulmonary arteries. The dorsal portion of each arch, on the other hand, is known as the *duct of Botallo* or *ductus arteriosus*, which, as will be noted below, atrophies at the time of hatching.

The Subclavian Arteries. — As noted under the description of external features the primordia of the anterior and posterior limb buds appear by the end of the day as broad swellings on the sides of the body. Correlated with this we find that on the fourth day the eighteenth segmental artery on each side gives rise to a branch which extends out toward the respective bud. It is the *primary subclavian artery*. From it, at the point where it enters the limb, a branch also extends anteriorly toward the third aortic arch. This is destined to form the permanent subclavian (see fifth day).

The Sciatic Arteries. — Posteriorly, a pair of segmental arteries also enlarge and grow out toward the *hind limb buds*. These vessels become

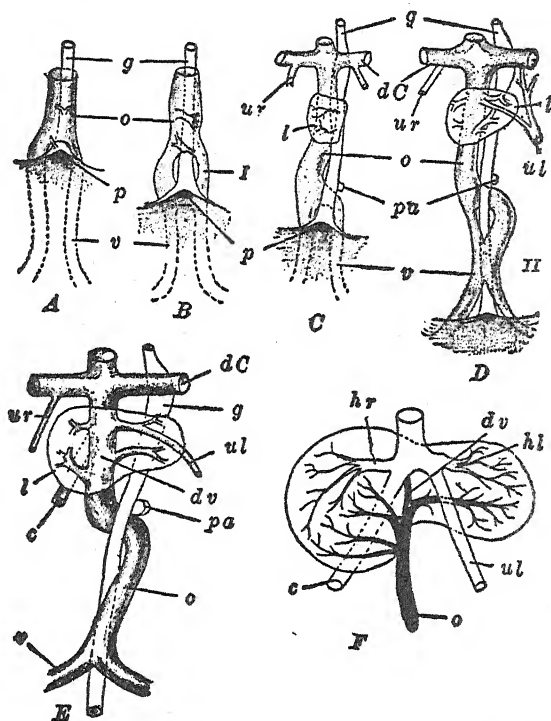


Fig. 211. — Diagrams illustrating the formation of the omphalomesenteric and umbilical veins, in the Chick, ventral view. From Kellicott (*Chordate Development*). After Hochstetter. A. At about 58 hours. B. At about 65 hours the veins are joined dorsal to the gut by a short transverse vessel. C. At about 75 hours the anterior intestinal portal has moved posteriorly somewhat so that the transverse vessel appears to be more anterior. At the same time, the left side of the loop, which its development created, has disappeared. D. At 80 hours a second loop has been formed by the fusion of the vitelline veins beneath the gut. E. At about one hundred hours the right side of this new loop has also disappeared. F. At about 130 hours, just before the disappearance of the main portion of the ductus venosus within the liver. This figure is obviously on a much smaller scale than E.

c. Vena cava posterior (inferior). dc. Ductus Cuvieri. dv. Ductus venosus. g. Gut. hl. Left hepatic vein. hr. Right hepatic vein. l. Liver. o. Omphalomesenteric or vitelline vein (the posterior continuation of the ductus venosus). p. Anterior intestinal portal. pa. Rudiment of pancreas. ul. Left umbilical vein. ur. Right umbilical vein. v. Vitelline vein. I, II. Primary and secondary venous rings around the gut.

the *sciatic arteries*, and as the legs develop they grow with and supply them.

The Umbilical Arteries. — During the fourth day, each sciatic artery gives off at its base a branch which extends into the allantois. These are the *umbilical* or *allantoic arteries*. Later (eighth day), the right member of this pair starts to disappear, while the left becomes a very important embryonic vessel, furnishing blood to the allantois. Indeed, so large does it become that the left sciatic seems for a time to be merely a branch from it.

The Renal Arteries and Those of the Gonads. — Numerous branches from the dorsal aorta supply the mesonephros at this time, and later on a few of these persist as the *renal arteries*. Branches from the aorta also supply the reproductive organs as these develop.

The Veins.

The Vitelline Veins. — It will be recalled that at the close of the third day, the vitelline veins within the embryo had been united by a transverse vessel dorsal to the intestine, so that the latter was surrounded by a venous ring. Between this time and the close of the fourth day, further changes have taken place in this region, as follows: Very shortly after the transverse vessel has been formed the left side of the above ring disappears (Fig. 211, C). Later, as the anterior intestinal portal moves backward, the vitelline veins between the portal and the transverse vessel fuse with one another beneath the intestine. In this manner, a venous ring is again formed around the posterior extremity of the fore-gut, and in this case the right side presently begins to grow smaller. Anterior to the vitelline veins the ductus venosus continues to receive capillaries from the surrounding liver (Fig. 211, D).

The Cardinal Veins. — The anterior cardinals, as indicated in the previous chapter, have, by this time, reached a stage when they may be known as jugulars, while the posterior cardinals continue as previously described. The subcardinals which started to form on the third day become distinct vessels and presently acquire several direct connections with the posterior cardinals lying on the dorso-lateral sides of the mesonephros (Fig. 212).

The Inferior or Posterior Vena Cava. — This important vessel of the adult Bird begins to develop at this time out of some of the capillaries in the dorsal part of the liver on the right side. Slightly further back it is also augmented by venous islands in a fold (the *caval fold*) of one of the liver mesenteries. These capillaries and venous islands soon fuse

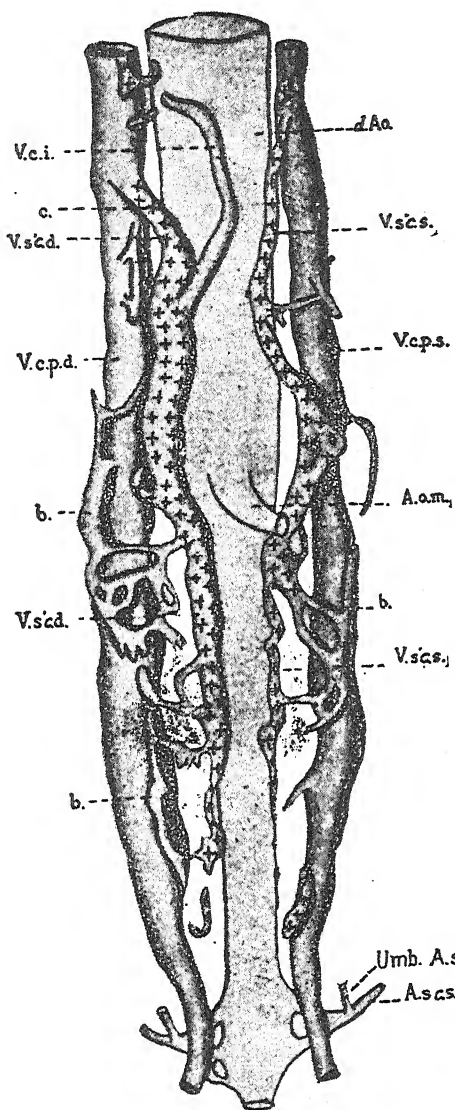


Fig. 212. — Reconstruction of the venous system of a Chick, 90 hours, ventral view. From Lillie (*Development of the Chick*). After Miller.

A.o.m. Omphalomesenteric (vitelline) artery. *a.s.c.s.* Left sciatic artery. *A.u.s.* Left umbilical artery. *b.* Vessels enclosed within ventral side of mesonephros. *c.* One of the direct connections of subcardinal with posterior cardinal. *V.c.p.d.,s.* Right and left posterior cardinal veins. *V.c.i.* Vena cava inferior. *V.sc.d.,s.* Right and left subcardinal veins.

together so as to form a definite vein which empties anteriorly into the ductus venosus (Fig. 211, *E*), and posteriorly establishes a connection with the right subcardinal (Fig. 212). Its subsequent development will be described in the following chapter.

The Umbilical Veins. — Upon the fourth day, the veins of the lateral body wall acquire connections with efferent vessels which have developed in the allantois, and at the same time, the right vein begins to disappear, along with the transitory subintestinal vein. The left vein on the other hand persists, but presently loses its anterior outlet into the ductus Cuvieri. At the same time, however, it develops new connections with the anterior half of the ductus venosus (Fig. 211, *D, E*). Through these, therefore, blood from the allantois flows quite directly into the latter vessel, without taking any extensive part in the hepatic portal circulation. Later, these connections with the ductus venosus fuse into one, which thus constitutes the anterior extremity of the single umbilical vein (Fig. 211, *F*). Eventually this vein acquires a median position in the embryo instead of its original lateral one. Subsequent to hatching, its proximal portion persists as a vein of the ventral body wall.

The Pulmonary Veins. — These vessels also develop at about this period in connection with the rudiments of the lungs, and presently become connected with the heart in the region of its left atrium.

EXTRA-EMBRYONIC BLOOD VESSELS

The Arteries. — During the fourth day the proximal portions of the vitelline arteries become fused with one another so as to leave the dorsal aorta as a single vessel. This fusion, however, occurs for only a relatively short distance, and never passes beyond the end of the umbilical stalk. From that point, the two main vessels continue to run out laterally, branching as they go, and terminating in a network of capillaries just inside the sinus terminalis. Subsequent development does not fundamentally alter the arterial plan except that as the septa of the splanchnopleure develop in the yolk-sac, the arterial capillaries come to occupy the deeper portions of these septa.

The Veins. — By the end of this day the right anterior vitelline vein has disappeared, while the left anterior vitelline vein and the posterior vein, are well developed. The lateral vitelline veins have also become larger and more definite at the point where they extend outward in company with the arteries. Further out in the area vasculosa, they continue to branch extensively, the branches connecting with the intermediate veins as already noted. By this time, however, these con-

nections are so pronounced that the intermediate vessels appear merely as the finer endings of the lateral vitellines, uniting these veins with the sinus terminalis (Fig. 182). Subsequent to the tenth day, the anterior and posterior vitelline veins are gradually eliminated, the lateral veins persisting as the main efferent vessels of the yolk-sac. After the tenth day, the sinus terminalis is no longer distinct, becoming obliterated by a mass of capillaries. These capillaries and the vessels with which they are connected, forming the area vasculosa, then continue to spread over the yolk in company with the yolk-sac mesoderm. Thus, like the latter, they come at last virtually to surround it.

THE NERVOUS SYSTEM

This system, like the others, continues to develop through embryonic life. The differences observed in it between the fourth and fifth days, however, are not, in most respects, very great. Therefore, since it is not proposed to carry a detailed chronological description of any of the organs beyond the fifth day, we shall conclude the account of the nervous system in the present chapter.

THE FLEXURES

The cranial and cervical flexures of the brain and nervous system have already been noted in the account of external changes through the fourth day. As has been indicated in the general discussion of this matter, only one of the flexures just named, i.e., the cranial, is permanent, the cervical gradually straightening out until it is entirely gone. Also, though to a smaller extent than in the Frog, even the cranial flexure is partly obscured in the adult brain by the development of the cerebral hemispheres and other parts. There is now to be noted a third flexure, which though barely visible on the fourth day, later becomes quite marked. It, like the cranial, is permanent and also like the cranial is never entirely obscured. This is the *pontine flexure* which consists of a ventral bulge in the thickened floor of the myelencephalon (Fig. 214).

THE PROSENCEPHALON

The Telencephalon. — The cerebral hemispheres continue to increase in size during the fourth day, and their lateral walls in particular, are thickening to form the *corpora striata*. The other features already noted as characteristic of this portion of the brain have also increased in prominence. As regards subsequent development the cere-

bral hemispheres ultimately become one of the most noticeable portions of the brain, their backward growth causing them to overlap, and to conceal partially the large optic lobes. Their surface, however, never attains the complicated convolutions so characteristic of the Mammal. Anteriorly, beginning about the eighth day, small portions of these hem-

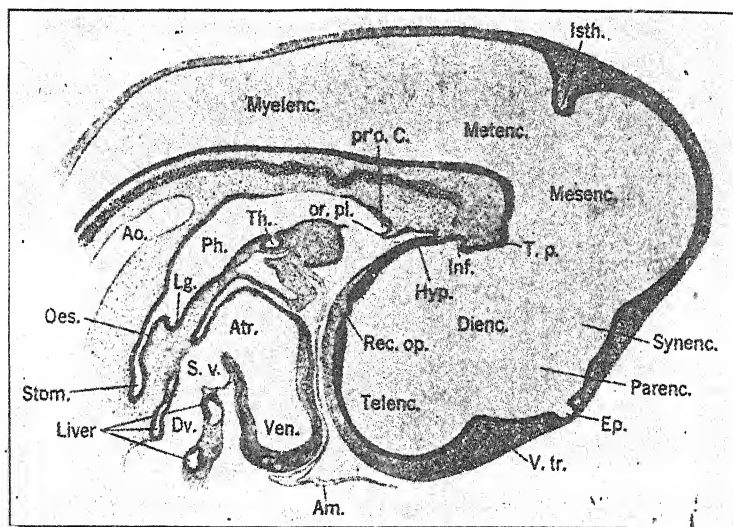


Fig. 213. — Optical longitudinal section of the head of an embryo of 39s. From Lillie (*Development of the Chick*).

Atr. Atrium. B.a. Bulbus arteriosus. D.v. Ductus venosus. Lg. Laryngotracheal groove. Oes. Oesophagus. or.pl. Oral plate, which has now ruptured. Parenc. Parencephalon. Ph. Pharynx. Stom. Stomach. Synenc. Synencephalon. Th. Thyroid. S.v. Sinus venosus. Ven. Ventricle. Other abbreviations as before.

ispheres become partially constricted away from the main posterior parts to form the *olfactory lobes*.

Concerning other parts of the telencephalon, as already indicated, the anterior commissure appears in the midst of the torus transversus. On the fifth day, also, an evagination develops at the antero-dorsal boundary of the lamina terminalis just between it and the velum transversum; it is the *paraphysis*. This structure virtually marks the boundary between the telencephalon and diencephalon, Lillie placing it in the former, and some anatomists in the latter. Above this body occurs the inward bend of the wall which constitutes the velum transversum, whose more dorsal half at least, according to most authorities, lies definitely in the diencephalon.

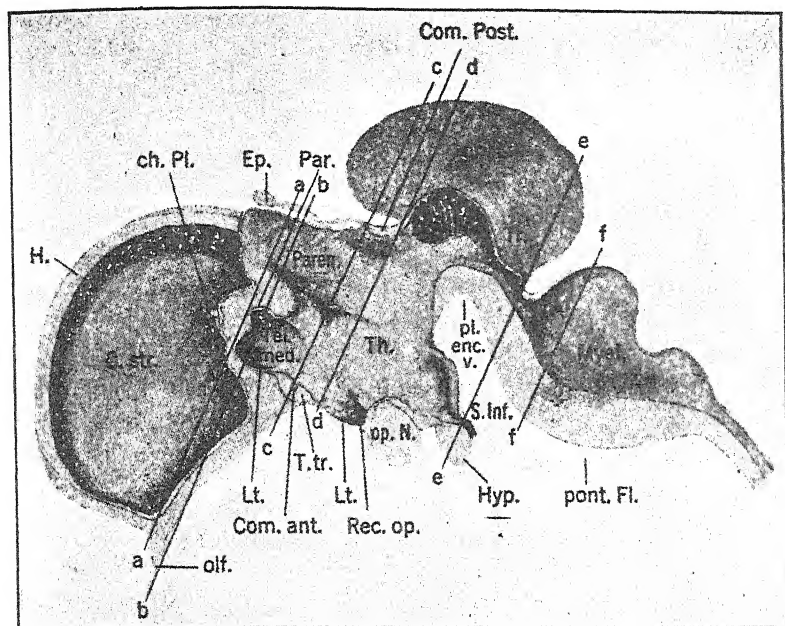


Fig. 214. — Dissection of the brain of an 8-day Chick. From Lillie (*Development of the Chick*). The arrows shown in the figure lie near the dorsal and ventral boundaries of the foramen of Monro.

ch.Pl. Choroid plexus (anterior). *Com.ant.* Anterior commissure *Com.Post.* Posterior commissure. *C.str.* Corpus striatum. *Ep.* Epiphysis. *H.* Hemisphere. *Hyp.* Hypophysis (anterior stomodaeal, part). *Lt.* Lamina terminalis. *Myel.* Myelencephalon. *olf.* Olfactory nerve. *op.N.* Optic chiasma. *op.L.* Optic lobe. *Par.* Paraphysis. *Paren.* Parencephalon. *pl.enc.v.* Plica encephali ventralis. *pont.Fl.* Pontine flexure. *Rec.op.* Recessus opticus. *S.Inf.* Saccus infundibuli. *Tel.med.* Telencephalon medium. *Th.* Thalamus. *T.tr.* Torus transversus. *Tr.* Commissura trochlearis.

The lines *a-a*, *b-b*, *c-c*, *d-d*, *e-e*, *f-f*, represent the planes of sections not figured in this text.

The wall of this portion of the fore-brain, therefore, gives rise to the anterior commissure and the cerebral hemispheres. Its cavity forms the anterior part of the third ventricle into which the lateral ventricles of the hemispheres open through the foramina of Monro.

The Diencephalon. — The anterior part of the roof in this region of the brain, as noted, apparently consists of the dorsal half of the velum transversum which later becomes folded to form the *anterior choroid plexus*. Eventually this plexus develops anterior branches extending forward into the lateral ventricles of the cerebral hemispheres. Posterior to the plexus the epiphysis shows no great change on the fourth day. Later, however, it grows out into a long narrow tube, whose

end is dilated and possessed of numerous buds, the *epiphysial* or *pineal gland*. Just posterior to this organ at the boundary between the fore- and mid-brains, the *posterior commissure* eventually develops within the broad constriction which has marked this point from the first.

During the fourth day no striking development occurs in the lateral

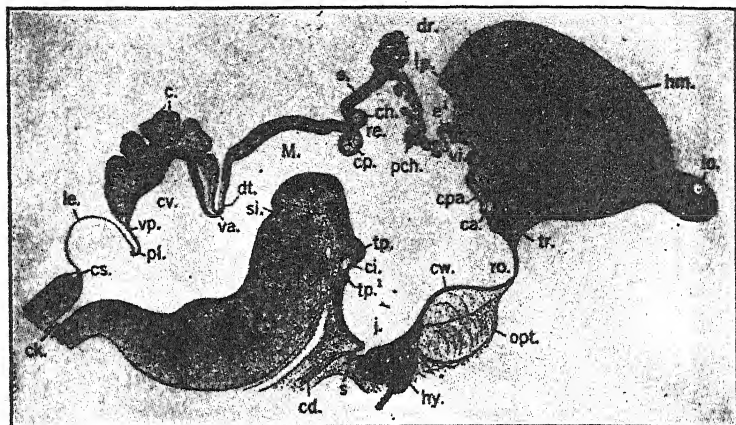


Fig. 215. — Median sagittal section through the brain of the Chick of 12 to 13 days. From Kupffer (Hertwig's *Handbuch*, etc.).

c. Cerebellum. ca. Anterior commissure. cd. Notochord. ch. Habenular commissure. ci. Infundibular commissure. ck. Central canal of spinal cord. cp. Posterior commissure. cpa. Anterior pallial commissure. cs. Spinal commissure. cv. Cavum cerebelli. cw. Optic chiasma. dr. Epiphysial (pineal) gland. dt. Decussation of the trochlear (IV) nerve. e. Epiphysis. e₁. Paraphysis. hm. Cerebral hemisphere. hy. Hypophysis (anterior part). j. Infundibulum. le. Ependymal lamina of the roof of the fourth ventricle. lo. Olfactory lobe. lp. Posterior lobe of cerebral hemisphere. M. Mesencephalon. opt. Optic chiasma. pch. Choroid plexus third ventricle. pl. Choroid plexus of fourth ventricle. re. Epiphysial recess. ro. Optic recess. s. Saccus infundibuli. si. Posterior intracephalic furrow. tp. Tuberculum posterius. tp₁. Tuberculum mammillare. tr. Torus transversus. va. Velum medullare anterius. vi. Median ventricle of telencephalon. vp. Velum medullare posterius.

or ventral region of the diencephalon. Subsequently, however, the former region becomes greatly thickened to form the *thalami*. On the ventral side, the fate of the infundibulum has already been described (see discussion of fore-gut, third day) while the *optic chiasma* comes to comprise a thick bundle of fibers from the optic nerves.

The floor of this posterior division of the fore-brain thus gives rise to the optic stalks, the optic chiasma and the infundibulum, while the optic thalami develop within the lateral walls. The roof forms the anterior choroid plexus and the epiphysis; the cavity constitutes the posterior part of the third ventricle.

THE MESENCEPHALON

There is nothing in particular to be said concerning the development of this region on the fourth day. Later we find that the growth and thickening of the dorso-lateral parts of the mid-brain greatly exceed that of a narrow dorso-median strip, thus producing the two large *optic lobes*, which the median strip separates from one another by a fissure. Ventro-laterally, the sides and floor of the mid-brain also become thickened, constituting the *crura cerebri*. This thickening finally results in narrowing the central canal to form the *aqueduct of Sylvius* or *iter*, which connects the cavities of the third and fourth ventricles.

THE RHOMBENCEPHALON

The Metencephalon. — The thickening which was noted in the roof of this region on the third day continues to increase, resulting finally in the production of a large median lobe, and two small lateral lobes united with it. The body thus formed extends backward somewhat so that it partially overhangs the myelencephalon. It is the *cerebellum*. About the ninth day, transverse fissures appear on the surface of this organ, which deepen as development proceeds. The ventro-lateral walls of the metencephalon, which have also been thickening, come eventually to form the *pons Varolii*.

The Myelencephalon. — It has already been stated that the roof of this region of the brain remains thin; it eventually forms the *choroid plexus* of the fourth ventricle. The ventral and ventro-lateral walls, however, showed signs of thickening on the third day. This tendency increases, and these walls finally constitute the *medulla oblongata*.

THE SPINAL CORD AND SPINAL NERVES

The description of the development of the cord and of the somatic spinal nerves was completed in Chapter 11. The completion of the sympathetic and parasympathetic systems, i.e., the *autonomic*, will now be noted.

The Sympathetic and Sacral Parasympathetic Systems. — It will be recalled that at the end of the third day the primary sympathetic and sacral parasympathetic systems had just been established. They consisted of two slender cords and their ganglia lying just dorso-lateral to the dorsal aorta, and extending from the region of the vagus ganglion to the tail. On the fourth and fifth days neuroblasts migrate from each ganglion of the primary systems to positions above the primary cords just median to where each somatic trunk divides (Fig. 216). Each such

aggregation of neuroblasts, or ganglion, forms neurons which again send axones anteriorly and posteriorly to form the *paravertebral* or *permanent sympathetic* and *sacral parasympathetic* cords. For a time both primary and secondary cords exist to some degree, but eventually the

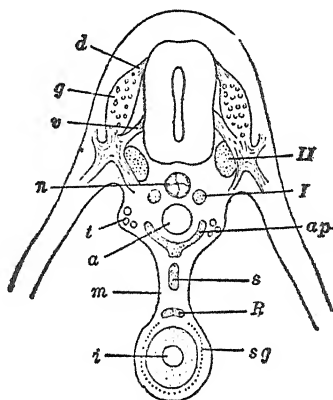


Fig. 216.—Diagram of the chief elements of the sympathetic nervous system of the Chick, in transverse section.—From Kellicott (*Chordate Development*). After His, Jr.

a. Dorsal aorta. op. Aortic plexus. d. Dorsal (afferent) root of spinal nerve. g. Spinal ganglion. i. Intestine. m. Mesentery. n. Notochord. R. Remak's ganglion. s. Splanchnic plexus. sg. Sympathetic elements in intestinal wall. t. Mesonephric tubules. v. Ventral (efferent) root of spinal nerve. I. Primary sympathetic cord. II. Secondary sympathetic cord. The rami communicantes are only partially shown.

side of the neural canal in the *nuclei of Terni*. From these, cell fibers grow out through the ventral somatic nerve roots to the points where these roots join their respective dorsal roots. The preganglionic sympathetic and sacral parasympathetic fibers then leave the somatic roots and through short connections, the *secondary* or *permanent rami communicantes*, enter the ganglia of the permanent sympathetic and sacral parasympathetic cords. Either in these ganglia (sympathetic) or in the ganglia of the visceral plexuses (parasympathetic) they synapse with the postganglionic fibers of these plexuses.

primary cord is mostly eliminated. It is generally thought that neuroblasts from the ganglia of the permanent cords also migrate to the mesentery and viscera to form the visceral plexuses, but, save for the sacral ganglia, Yntema, '55 denies this, and claims that in the Chick at least, all these visceral plexus neuroblasts are from the vagus crest (see below). Though unorthodox this view is supported by extensive investigations.

It should be emphasized at this point that all the neurons so far described as originating from the neural crests, constitute only the postganglionic elements of the systems under discussion. The preganglionic neurons on the other hand are all derived from neuroblasts in the neural tube. These cells at first occupy the ventro-lateral parts of the tube along with the somatic motor neurons. From here the sympathetic and sacral parasympathetic neuroblasts separate from the somatic neuroblasts, and migrate dorsally taking up positions on either

It should now be noted that all the nerves and fibers of the autonomic system, i.e., the sympathetic and sacral parasympathetic already discussed, and the cranial parasympathetic described below, are strictly motor. Nevertheless there are sensory fibers which convey sensations from the viscera. These arise from neurons in the cranial and spinal ganglia where all sensory neurons outside the nose, eye, and ear are located. They leave the dorsal roots through the rami communicantes and accompany the motor fibers of the autonomic system to the viscera, though not part of that system.

The Cranial Ganglia, Mixed Nerves, and Cranial Parasympathetics. *Trigeminal Ganglion and Nerve.*—It has been stated that this ganglion has the form of an inverted Y. On the fourth day fibers from the anterodorsal branch, i.e., the *ophthalmic*, pass anteriorly along the dorsomedian wall of the optic vesicle. Eventually these ophthalmic fibers, mostly sensory, reach the face and beak. The other branch of the Y extends toward the angle of the mouth, where it also divides, one part, the *mandibular*, is a mixed nerve, and supplies the lower jaw. The other all sensory branch, the *maxillary*, supplies the upper jaw. As usual all sensory fibers arise from neurons in the ganglion, while the motor fibers are from neurons in the brain.

The *Acustico-facialis* Ganglion and Nerves.—As indicated above, the ganglion which gives rise to the VII and VIII nerves is at first in a single mass. During the fourth day, however, the antero-ventral portion separates from the remainder, and gives rise to a nerve which extends chiefly along the hyoid arch, though possessing also a small branch to the mandibular. This is the rudiment of the future VII or *facial nerve* with a motor component from the medulla. The remainder of the ganglion gives rise to the VIII or *auditory nerve* which is purely sensory, and which communicates with the inner ear as described below.

The *Glossopharyngeal* Ganglion and Nerve.—The origin of the IX cranial ganglion was noted in the account of the second day, where it was indicated as lying above the third arch. The IX nerve appears on the fourth day and extends into this arch. Later another branch enters the second arch, and together they eventually supply the tongue and pharyngeal region.

The *Vagus* and Cranial Parasympathetic System.—Neuroblasts in the crest and an adjacent placode above the third branchial pouch, together with neuroblasts within the brain, produce the vagus complex as follows: Upon the fourth day the crest part of the X ganglion separates from the placodal portion, and eventually produces the *ganglion jugu-*

lare, the placodal part producing the *ganglion nodosum*. The exact origin of all the neural elements of the X nerve complex in the Chick is still uncertain, but the situation seems to be thus: Neuroblasts of the ganglion jugulare produce the somatic sensory neurons, the somatic motor neurons arising from within the medulla. The crest produces all postganglionic neurons of the cranial parasympathetic system (Yntema and Hammond, '55) except possibly those of the ciliary ganglion, said by Levi-Montalcini and Amprino, '47, to be derived from mesenchyme; the preganglionic neurons of this system arise within the medulla. From the ganglion nodosum nerves pass into the fourth and fifth neural arches and posteriorly to the heart, lungs, stomach, and intestine, while the ganglion moves back into the thorax. Eventually a part of the nodosum is detached as the *ganglion cervical primum*.

THE CRANIAL MOTOR NERVES

The Motor-ocular or III Nerve. — The early development of this cranial motor nerve has already been described. During the fourth day, it passes down beneath the optic stalk, and there enters a ganglion. This receives a connection from the ophthalmic branch of the V nerve, and is known as the *ciliary ganglion*. The III nerve ends by innervating the superior, inferior, and internal rectus, and the inferior oblique muscles of the eye when these develop.

The IV or Trochlearis Nerve. — This motor nerve does not appear until the fifth or sixth day, but will be described at this point. It is peculiar as a motor nerve, in that it arises from the dorsal side of the brain, at the bottom of the isthmus. It has no connection with any ganglion, and ultimately innervates the superior oblique eye muscles.

The VI or Abducent Nerve. — This is a perfectly typical motor nerve, appearing toward the end of the fourth day. It has no ganglion, and arises from the ventral side of the medulla median to the point of origin of the fifth nerve. It innervates the external rectus muscle of the eye.

The XI or Spinal Accessory Nerve. — There is no data on the development of this nerve in the Chick (Lillie).

The XII or Hypoglossus Nerve. — This nerve develops during the fourth day from two pairs of ventral roots on the medulla at the level of the third and fourth somites. There are no ganglia, and the roots are evidently serially homologous with the ventral roots of the spinal nerves. The nerve to which they give rise eventually innervates the floor of the pharynx.

THE ORGANS OF SPECIAL SENSE

THE EYE

At the end of the third day the inner wall of the optic cup had thickened, and the whole cup was in the process of enlarging. The lens, meanwhile, had separated from the external ectoderm, and the side of the lens toward the cup had also begun to thicken. The further development of the eye may be described as follows:

Parts Connected with the Optic Cup. — During the fourth day, pigment begins to appear in the wall of the optic cup nearest the brain, i.e., its outer wall. At the same time, there is developing upon the innermost surface of the inner wall, the *internal limiting membrane*. Beneath this membrane, but still toward the inner side of the inner wall, as noted on the second day, neuroblasts near the fundus have sent out axones. These have passed over the retinal elements just beneath the limiting membrane, and have reached the optic stalk through the proximal part of the choroid fissure. Here they proceed among cells of the ventral wall of the stalk, and late on the fourth or early on the fifth day, reach the brain and form the *optic chiasma*. Later many more-fibers grow through the ventral part of the optic stalk, causing it to swell so that the original internal cavity is obliterated. It may then be termed the *II* or *optic nerve*. In this connection it may further be noted that during the fifth and sixth days the processes of growth occur in such a manner as to alter the relative position of the point of attachment of the optic stalk to the cup. The result is that at the completion of these processes the point in question is no longer at the ventral edge of the cup, but approximately at its center, opposite to the lens.

Subsequent to the fourth day, other changes are also occurring in the walls of the optic cup. As the various cell layers of the retina are formed in the inner wall, this wall shows differentiation into two zones. The central and larger of these, which includes the fundus, is called the *retinal zone*, i.e., the *retina* proper, and it is only within this zone that the above retinal elements are developed. The remainder of the inner wall consists merely of a band around the rim of the cup, and is known as the *lenticular zone*. The line of separation between the two is known as the *ora serrata* (Fig. 217). Within the retinal zone, the outer wall forms the *pigmented layer* of the retina, but never completely fuses with it. In the lenticular zone, on the contrary, fusion between inner and outer walls is complete, pigment penetrates them both, and both remain

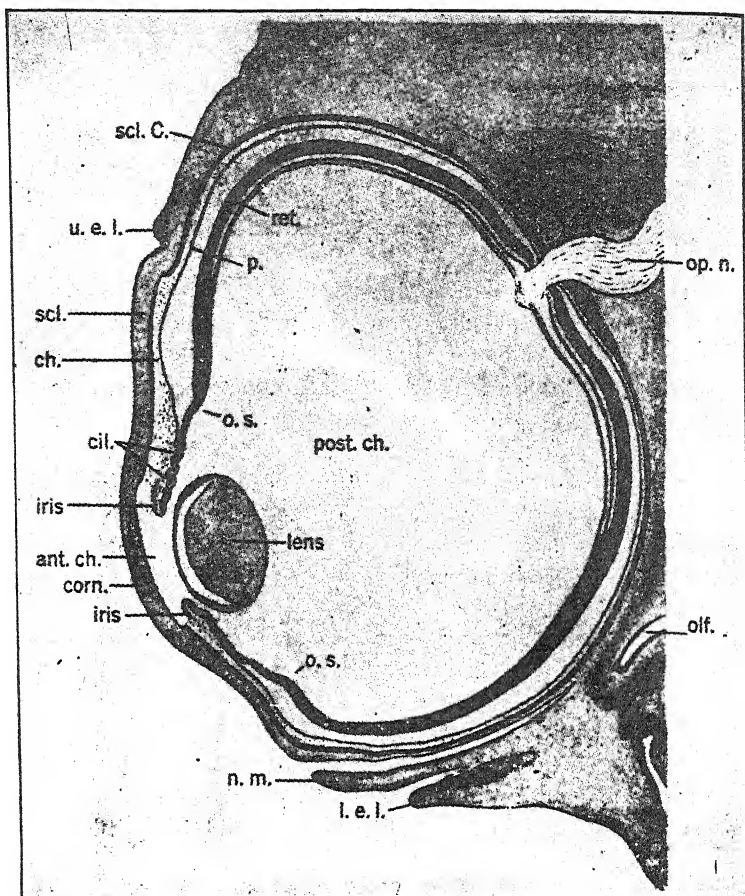


Fig. 217. — Frontal section of the eye of an eight-day Chick. From Lillie (*Development of the Chick*).

ant.ch. Anterior chamber of the eye. *ch.* Choroid coat. *cil.* Ciliary processes. *Corn.* Cornea. *l.e.l.* Lower eyelid. *n.m.* Nictitating membrane. *olf.* Olfactory sac. *op.n.* Optic nerve. *o.s.* Ora serrata. *p.* Pigment layer of the optic cup. *post.ch.* Posterior (vitreous) chamber of the eye. *ret.* Retina. *scl.* Sclerotic coat. *scl.C.* Sclerotic cartilage. *u.e.l.* Upper eyelid.

relatively thin. From this zone, in connection with certain mesenchymal elements, are differentiated the *iris* and the *ciliary processes*. While these parts are forming, the cavity of the optic cup is being filled with a gelatinous matrix containing fibers. Both elements are probably derived from certain cells of the retinal and lenticular zones, and together are known as the *vitreous humor*. Certain of the fibers of the humor are con-

nected with the ciliary processes, and help to support the lens. Finally, the outside of the cup is gradually covered by two layers of mesenchymal origin. The inner is the *choroid coat*, and the outer the *sclerotic coat*, the latter being partly cartilaginous.

The Pecten.—This body is also developed in connection with the optic cup and choroid fissure, but is entirely peculiar to the Birds. It

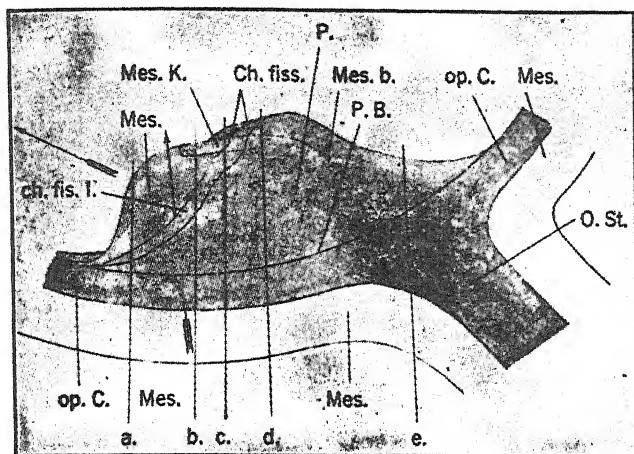


Fig. 218.—Diagrammatic reconstruction of the pecten of the eye of a Chick embryo of 7½ days' incubation. From Lillie (*Development of the Chick*). After Bernd.

Ch.fis.l. Lip of the choroid fissure. *Ch.fiss.* Choroid fissure. *Mes.* Mesenchyme. *Mes.b.* Upper edge of the mesenchymal ridge covered by the lips of the choroid fissure. *Mes.K.* Thickening of the edge of the mesenchymal ridge. *op.C.* Optic cup. *O.St.* Optic stalk. *P.* Pecten. *P.B.* Base of the pecten.

The arrow indicates the direction of growth of the lips of the choroid fissure over the mesenchymal ridge. The line *d* shows the plane of the section reproduced in Fig. 219.

has seemed well, therefore, to emphasize it by a separate description. It arises during the fourth day in the form of a blood vessel embedded in mesenchyme. This mesenchymal mass is in the shape of a ridge which enters the cavity of the cup through the choroid fissure near its proximal end. The distal end of the fissure between this mesenchyme and the rim of the cup has, meanwhile, been closed. On subsequent days, the mesenchymal ridge pushes up into the cavity, while at the same time it is being gradually covered over by the in-turning and up-growth of the edges of the choroid fissure on either side of it. This covering soon becomes more prominent than the relatively thin ridge of mesenchyme

which it has overgrown, and presently (eighth day) the two parts become indistinguishable. Though remaining constricted at its base, the ridge of fused tissues inside the cavity of the cup continues to grow somewhat, and later becomes folded, assuming the appearance of a fan, though in most Birds it is more comb-like, and hence is named the *pecten*. It is very vascular and probably helps to nourish the retina. The opening in the choroid fissure between pecten and optic stalk provides

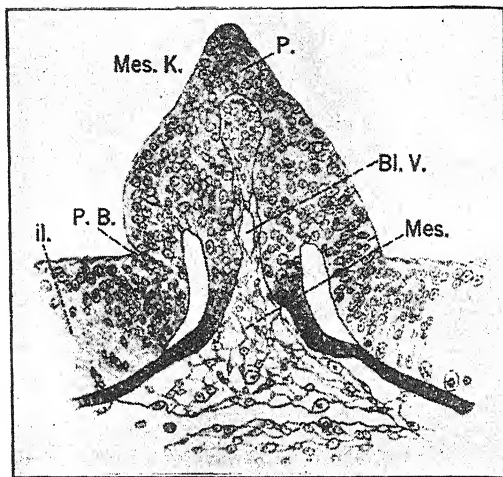


Fig. 219.—Section in the plane of *d.* of Fig. 218. to show the histological structure. From Lillie (*Development of the Chick*). After Bernd. *Bl.v.* Blood vessel in mesenchymal ridge. *il.* Retinal layer of optic cup. Other abbreviations as in Fig. 218.

the exit for the optic nerve fibers from the retina. A few of these fibers run directly to this point, but the majority come to the base of the pecten, and run along its sides to the place of exit (Figs. 218 and 219).

The Lens.—At the end of the third day, the inner wall of the lens vesicle had thickened considerably by virtue of the lengthening of its cells. This process continues for several days until the cavity of the vesicle is entirely obliterated. Moreover, inasmuch as the lengthening of the central cells is greater than that of those at the periphery, the inner surface of the lens becomes distinctly convex (Fig. 217). These lengthened cells of the inner wall form the core of the future lens, while the cells of the outer layer toward the ectoderm form a simple flat epithelium. The lens now grows, largely by the production of cells at its equator where the inner and outer walls meet. These cells become fiber-like

and wrap themselves around the original elements which form the core, thus increasing the size of the lens by the addition of concentric layers of cells.

The Cornea, the Anterior Chamber, and the Lids.—The cornea at first consists merely of the external ectoderm opposite the lens. On the fourth day, however, this layer is augmented internally by a thin non-cellular layer of mesenchymal origin. On the fifth day, this thickens slightly, and begins to be covered on the side toward the lens by a third layer formed of mesenchymal cells. Later, the middle layer becomes cellular by the migration into it of cells from the mesenchyme, while the third and innermost layer forms a typical epithelium. The latter finally becomes continuous at its edges with the cells of the sclerotic coat. The *cornea* thus constituted arches outward slightly, and thus a chamber is formed between its inner layer and the front of the lens. This is the *anterior chamber*, and it becomes filled with the *aqueous humor*. The *lids* begin to develop about the seventh day as folds of the integument surrounding the cornea (Fig. 217).

THE EAR

The Internal Ear.—At the end of the third day, the otocyst, or future internal ear, was in the form of a sac. The uppermost portion of the sac had been slightly constricted away from the lower major portion, and had started to grow upward somewhat as the rudiment of the endolymphatic duct. This upper portion, furthermore, still retained its narrow tubular connection with the exterior (Fig. 206). There is, in these parts, no marked change characteristic of the fourth day. Upon the fifth day, however, the connection of the endolymphatic duct with the exterior is entirely lost. Moreover, the opening of the duct into the sac is being gradually shifted ventrally along the median side of the latter. At the same time, the dorsal part of the duct is continuing to grow upward, and expanding to form the *saccus endolymphaticus*. Eventually, this becomes embedded in mesenchyme above the hind-brain.

While these events are taking place in connection with the formation of the endolymphatic duct the remaining major portion of the otocyst is developing further, as follows: Upon the early part of the fifth day, there arises from its dorsal half a vertically elongated, hollow out-pushing in the direction of the ectoderm. Then a horizontal out-pushing appears just beneath the first, and therefore at about the equator of the otocyst. Presently a vertical split develops in the ventral part of the vertical out-pushing and soon extends dorsally, thus dividing it into an

anterior and a posterior ridge. The anterior, posterior, and horizontal ridges which have thus arisen are the rudiments of the respective *semicircular canals*. These canals eventually develop by a gradual constricting away of the hollow ridges, so that they become separated from the

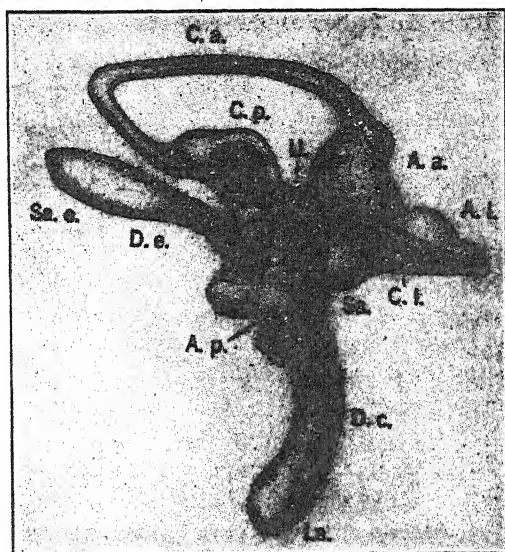


Fig. 220. — Model of the auditory labyrinth of the right side of a Chick embryo of 8 days and 17 hours; external view. From Lillie (*Development of the Chick*). After Röthig and Brugsch.

A.a. Ampulla of the anterior vertical semicircular canal. A.l. Ampulla of the lateral horizontal semicircular canal. A.p. Ampulla of the posterior vertical semicircular canal. C.a. Anterior vertical semicircular canal. C.l. Lateral horizontal semicircular canal. C.p. Posterior vertical semicircular canal. D.c. Ductus cochlearis. D.e. Endolymphatic duct. L.a. Lagena. S.a.e. Endolymphatic sac. U. Utriculus (utricle).

otocyst everywhere except at their ends. During this process a dilation occurs on each canal to form its *ampulla*. The remainder of the dorsal portion of the otocyst into which the canals open is the *utricle*.

Meanwhile, most of the ventral part of the otocyst has grown downward and also turned backward and toward the median line of the head. Its end forms the *lagena*, and the portion connecting this with the utricle is the *ductus cochlearis* or *cochlear duct*. The *sacculus* arises about the seventh day as a pouch on the median side of the uppermost portion

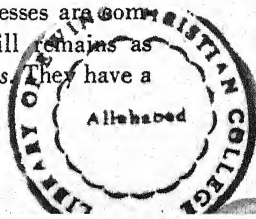
FOURTH DAY: ORGANS OF SPECIAL SENSE 423

of the ventral part of the otocyst, i.e., just above the point where the latter receives the ductus cochlearis (Fig. 206, B).

The parts of the inner ear thus far described constitute the *membranous labyrinth* (Fig. 220). The walls of this labyrinth are composed of epithelium, and its cavity is soon filled with the *endolymphatic fluid*. Except for small areas within the ampullae and at certain other points, the above epithelium becomes flat. At these points, however, elongated sensory cells end in hairs which project into the fluid, and among these cells grow the endings of nerve fibers (axones) coming from the VIII cranial ganglion.

On the sixth day, the mesenchyme which immediately surrounds the developing labyrinth begins to form a membrane (*membrana propria*) in close contact with it. At the same time the more peripheral mesenchyme is forming a cartilaginous case, separated slightly from the labyrinth and its membrane, but following all its contours. The space between the two is called the *perilymphatic space*. It is bridged by tissue which carries the nerves and blood vessels, and is filled by the *perilymphatic fluid* derived from loose mesenchyme tissue left within the space. The cartilaginous case later becomes ossified, and is known as the *bony labyrinth*. In it, on the side toward the middle ear, are two small openings, the *fenestra ovalis*, and the *fenestra rotunda*.

The Middle Ear, or Tubo-tympanic Cavity. — As was stated in connection with the alimentary tract, the first visceral clefts are closed during the fourth day, and the ventral portion of the pouch of each disappears. The dorsal portion, however, grows up toward the respective otocyst, and during the fifth and sixth days comes between it and the external epithelium. Each pouch then starts to enlarge, and the space within it is the dorso-lateral portion of one of the two *tubo-tympanic cavities*. Meanwhile, beginning on the fourth day, the ventro-median portion of each cavity is developed, as follows. In the antero-dorsal region of the pharynx, a horizontal shelf has grown backward, so as to produce a dorsal chamber virtually separate from the space beneath. Laterally, the part of each tubo-tympanic cavity already developed opens into this newly constituted dorso-median chamber. Then, as growth proceeds, an increasing portion of this chamber becomes drawn out into the respective cavities. Thus eventually the larger part of each middle ear space is really developed in this manner, rather than directly from the original "gill" pouch. When these processes are complete the median part of the dorso-median chamber still remains as such, while its lateral parts constitute the *Eustachian tubes*. They have a



common opening into the mouth by a single median slit-like aperture in the horizontal shelf. With regard to the cavities themselves two other points remain to be noted. First as in the case of the Frog, each tubo-tympanic cavity contains a bone, the *columella*. Its development can best be described, however, in connection with the tympanum. Secondly there is the peculiar relation which exists between the tubo-tympanic cavities and certain of the other bones of the Bird's skull. These bones like bones in other parts of the Bird skeleton to be described later contain spaces which give lightness to the body. The case of the head bones is noteworthy at this point, however, because in some of them the spaces are formed and filled by outgrowths from the tubo-tympanic cavities (Bremer, '40).

The External Auditory Meatus and the Tympanum. — It will be recalled that the temporary external opening of the first visceral pouch occurs only at its dorsal end. Ventrally, however, there is a fusion with the ectoderm which causes the latter to form a vertically elongated pit. When the dorsal perforation closes, that point also is marked by a pit. These pits presently disappear, and on the sixth day the point between them becomes marked by a new depression, the beginning of the *external auditory meatus*. It gradually deepens until, except for a thin layer of mesenchyme, the external ectoderm is in contact with the endoderm of the tympanic cavity. These thin layers of ectoderm, mesenchyme, and endoderm which thus separate the middle ear from the outside, constitute the *tympanum* or ear drum.

To the inside of the tympanum of an adult Bird is attached one end of the *columella*. The other end is in contact with a membrane covering the fenestra ovalis of the bony labyrinth, i.e., the bony case which finally surrounds the membranous labyrinth. The *columella* is, therefore, like a bridge stretching across the tympanic cavity from the tympanum to the inner ear. It is chiefly developed from mesenchyme which lies in the dorsal wall of the enlarged tubo-tympanic portion of the gill pouch. This mesenchymal rudiment, it may be noted, is thought to be derived from the dorsal end of the second or hyoid arch. However that may be, as the cavity increases in size, it extends upward on each side of the above mesenchyme until it has surrounded it except at its inner and outer ends. Then as this mesenchyme becomes cartilaginous and finally ossifies, it forms a bone (the *columella*), occupying the position already described. Lastly, it should be added that the inner end of this bone in contact with the membrane of the fenestra ovalis seems to arise, at least in some Birds, from an element (the *stapes*) which, though at first dis-

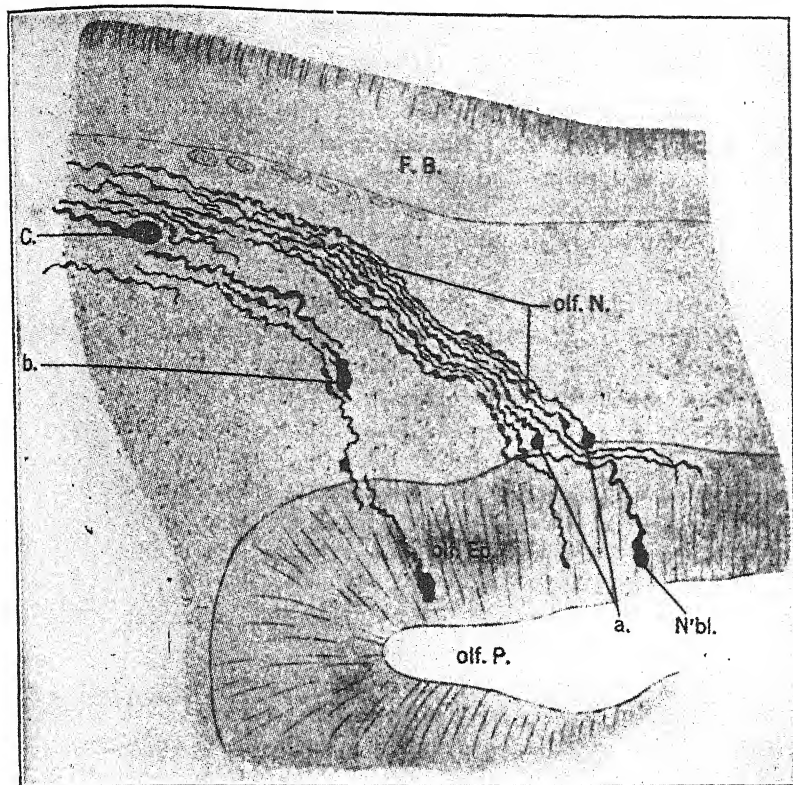


Fig. 221. — Sagittal section through the head of a Chick embryo of 5 days, showing the floor of fore-brain, olfactory pit, and developing olfactory nerve between. From Lillie (*Development of the Chick*). After Disse.

a. Unipolar neuroblasts near the olfactory epithelium. b. Bipolar cell in the olfactory nerve. c. Unipolar cell near the brain. F.B. Floor of fore-brain. N'bl. Neuroblast in the olfactory epithelium. olf.Ep. Olfactory epithelium. olf.N. Olfactory nerve. olf.P. Cavity of olfactory pit.

tinct, eventually fuses with the columella. This stapedial element in the Bird would thus apparently correspond to the opercular element in the ear of the Frog.¹

The Olfactory Organs. — It will be recalled that, at the close of the third day, the olfactory epithelium consisted of two types of cells:

¹ Some writers recognize a third element, the *stylohyal*, which enters into the formation of the columella of Birds. It must be stated, however, that the exact origins, as well as the homologies of the bones of the middle ear in the various groups of Vertebrates are not yet completely known.

simple epithelial cells and germinal cells. It had also become depressed to form the olfactory pits. During the fourth day this process of depression continues to a considerable extent, and thus the specialized olfactory epithelium lying at the bottom of the pits is carried in some distance from the surface. The epithelium forming the sides of the pits, on the other hand, is unmodified and similar to that outside. The position of the pits has also shifted somewhat with the growing of the head, so that their mouths now lie just on the antero-lateral border of the oral cavity.

At the same time that these processes are taking place, the germinal cells referred to are transformed into neuroblasts, and the latter in turn into typical neurones. On the external side, these neurones send short processes to the surface of the olfactory epithelium. On the other side, they produce axones which extend in toward the brain, the region of whose future olfactory lobes they do not enter, however, until about the sixth day. Along the course of these axones are a few bipolar neurones and also numerous epithelial cells, the latter serving as supporting and sheath cells for the fibers. Both types are said to migrate from the olfactory epithelium, to their final position during the growth of the axones. The axones, together with the other cells just indicated, constitute the *I cranial nerve* (Fig. 221).

On the fifth and succeeding days, the nasal cavities continue to deepen somewhat, and become greatly modified in shape. This is partly the result of the appearance of certain folds in the nasal wall; these folds are the rudiments of the three nasal turbinals, only two of which are finally covered by epithelium of the olfactory type.

While the internal development of the olfactory organ is thus progressing, certain external changes are also going on in connection with the apertures. However, since these changes have more to do with the development of the face than with that of the olfactory organs proper, they will be discussed under the heading of general external changes in Chapter 13.

THE URINOGENITAL SYSTEM

THE EXCRETORY SYSTEM

The Mesonephros. — At the end of the third day the pronephros had virtually disappeared, while the typical mesonephros was beginning to develop, posterior to the twentieth somite. During the fourth day, the

FOURTH DAY: THE REPRODUCTIVE SYSTEM 427

primary mesonephric tubules are developed from the most ventral vesicles throughout the greater part of the mesonephric region. The remaining vesicles which occur in every mesonephric segment are, moreover, each giving rise to a tubule. Thus besides the primary tubules, there are formed eventually *secondary* and *tertiary* tubules and sometimes even more, all of a similar nature, developing from the nephrotomal mass opposite each somite. As suggested in the previous chapter, the primary tubules thus formed soon connect directly, through a non-secretory or conducting portion, with the Wolffian duct. The others as they develop empty into outgrowths from that duct, which receive the name of *collecting tubules* (Fig. 207).

At the time that these tubules are developing, the remaining portion of each vesicle is forming a *Malpighian body* or corpuscle consisting of a glomerulus and its capsule. These Malpighian corpuscles are similar in essential respects to those found in the Frog, and need not be described further. Though its development is still incomplete, the mesonephros apparently starts to function as a kidney at this time (Boyden, '24). In this connection it is of interest to note that in the Bird a few of the more cephalic mesonephric tubules also establish rudimentary nephrostomal relations with the coelom in the manner characteristic of all these tubules in the Frog.

The Metanephros.—The rudiment of the *ureter* and collecting tubules of the *metanephros*, or permanent kidney of the Chick appears at the end of the fourth day as a diverticulum from the mesonephric duct. It arises from the dorsal side of this duct just at the point where the latter bends to enter the cloaca. During the fourth day, also, the nephrotomal tissue, just posterior to the thirtieth somite or end of the mesonephros, begins to degenerate for a short distance (see Chapter 13, Fig. 240). Thus anterior to this point, the mesonephros, and any undifferentiated nephrogenous tissue overlying it, become entirely cut off from the nephrotomal tissue posterior to this region. The latter tissue thus cut off accompanies the forward growth of the ureter and its collecting tubules, and is destined to form the secreting portion of the entire metanephros (see Chapter 13, Fig. 240).

THE REPRODUCTIVE SYSTEM

The Gonads.—The rudiments of the two gonads appear on the fourth day as thickenings of the peritoneal epithelium on each side of the dorsal mesentery, between it and the respective mesonephros. These thickenings occur just posterior to the origin of the vitelline arteries

and extend for seven or eight somites, i.e., through the posterior half or third of the mesonephric region. Presently primordial germ cells appear in this epithelial tissue, near to which they have been transported from the anterior part of the germ wall, where they are said to be distinguishable as early as the primitive streak stage. According to the remarkable observations of Swift ('14) and Goldsmith ('28) they are conveyed to their new location by the blood stream. No sex differentiation is apparent at this time.

The Gonoducts.—The future male gonoducts or *vasa deferentia* are the mesonephric ducts whose development has already been described.

The *oviducts* or *Müllerian ducts* begin their development at this time in both sexes in the form of two ridges, the *tubal ridges*. Each ridge is a strip of thickened peritoneum which appears on the fourth day. It lies on the dorso-lateral face of each mesonephros next to the body wall and near to the Wolffian duct. It is first found at about the level of the twentieth somite; from this point it differentiates posteriorly (see Chapter 13, Fig. 246)

THE ADRENALS

These bodies, though not really a part of the renal system, are closely connected with it, and their development may, therefore, best be described at this point.

As in the Frog, the adrenal organs are composed partly of cells derived from the peritoneum, and partly of cells from the sympathetic nervous system. The former element, known as the *cortical substance*, arises from the coelomic epithelium slightly anterior to the germinal region, and proliferations of this substance presently penetrate the mesenchyme between the Wolffian body and the dorsal aorta. The element derived from the sympathetic nervous system (mainly the primary sympathetic system) is known as the *medullary substance*, which comes into contact with the cortical material by the end of the fourth day (Willier, '30).

SUMMARY OF THE CONDITION AT THE END OF THE FOURTH DAY OF INCUBATION

I. GENERAL APPEARANCE

The *cervical flexure* has increased so that its mid-region is anterior and the diencephalon faces posteriorly. The *caudal flexure* has also in-

creased, and the embryo between it and the end of the cervical flexure is virtually straight. The entire embryo is on its side, and the *limb buds* have increased in prominence.

II. THE SOMITES

The number of pairs of *somites* has increased to forty-two, including all those which take part in the formation of adult structures, while the *myotomal*, *dermatomal*, and *sclerotomal* elements have been developed in each pair. The last named element forms a nearly complete sheath about the nerve cord and notochord, and shows slight indications of the *vertebral segments*. The account of the further development of the myotomal and dermatomal elements is completed in this chapter.

III. THE ALIMENTARY TRACT

The Fore-gut. — The rudiments of the *tongue* have appeared. The *first* and *second visceral clefts* have closed, and the third opened; the *visceral arches* reach their maximum development as such. The *thyroid* has completely separated from the floor of the pharynx. Subsequent development of the tongue and thyroid are indicated in this chapter.

The posterior end of the *laryngotracheal groove* and the *lung rudiments* have separated from the alimentary tract.

The *esophagus*, the *stomach*, and the *duodenum* have increased in length, and the two latter parts of the tract have developed a curve to the left. The *liver* has increased in size and come to lie somewhat in the curve of the stomach. The *dorsal pancreatic rudiment* has become a solid outgrowth and a pair of *ventral pancreatic rudiments* have arisen from the *ductus choledochus*. The *spleen* (not really a part of the alimentary tract) has started to develop.

The Mid-gut. — The mid-gut or region of the *small intestine* is now a virtually straight tube open to the yolk only by the relatively constricted aperture of the *yolk-stalk*.

The Hind-gut. — The anterior portion of the hind-gut constitutes the *rectum*, while its terminal portion becomes the *cloaca*. The latter is still separated from the exterior by the cloacal membrane, and its posterior part is laterally compressed.

IV. THE CIRCULATORY SYSTEM

The Heart. — The *ventricular* region, especially the transverse portion, has expanded and moved posteriorly. The *bulbus arteriosus* has swung toward the median line, and the *atrium* has rotated forward. The *interventricular*, the *interatrial*, and the *cushion septa* are developing.

The Embryonic Arteries.—The *second aortic arches* have disappeared, and the *fifth* and *sixth* pairs have developed. From the latter have arisen the roots of the *pulmonary arteries* which grow out and connect with the rudiments coming from the lungs. The *primary subclavian*, the rudiment of the *permanent subclavian* and the *sciatic arteries* have appeared, while the last named have given rise to the *umbilical* or *allantoic arteries*. The history of the sciatic and allantoic vessels is concluded in this chapter.

The Embryonic Veins.—The ring about the alimentary tract, which is formed in connection with the *vitelline veins*, has been broken by the disappearance of its left half. A fusion of the above vessels has occurred beneath the fore-gut, forming a second ring. The capillaries of the *ductus venosus* among the branches of the liver diverticula are becoming more numerous. Posteriorly, on the ventral side of the mesonephros, the rudiments of the *subcardinals* have become distinct vessels and have acquired direct connections with the posterior cardinals. The *inferior vena cava* has begun to form in the liver and caval fold, and posteriorly has connected with the right subcardinal. The longitudinal vein in the right body wall is disappearing, along with the transitory *subintestinal* vein, and the left, having acquired a connection with the allantoic vessels, has become the functional *umbilical vein*. The account of its development is completed. The *pulmonary veins* appear in connection with the developing lungs.

The Extra-Embryonic Arteries.—The *vitelline* arteries have fused with one another for a short distance as they leave the aorta. Their branches in the area vasculosa continue to develop in company with the growth of that region, but are without features requiring further note.

The Extra-Embryonic Veins.—The *right anterior vitelline vein* has disappeared, but the *left anterior*, *posterior*, and *lateral* veins are well developed. Subsequent development of the extra-embryonic veins is included in this chapter.

V. THE NERVOUS SYSTEM

The Brain.—The *cranial* and *cervical flexures* have increased slightly; the *pontine* flexure may be in evidence. The *cerebral hemispheres* have increased in size, and their lateral walls are thicker. The *optic lobes* are also becoming steadily more prominent. There are no other marked changes evident at this time.

The Spinal Cord and Spinal Nerves.—There is no special development on the fourth day.

The Cranial Ganglia and Mixed Nerves. — From the *V nerve ganglion* a branch (*ophthalmic*) has extended toward the future beak and another (*mandibular*) toward the angle of the mouth. The *VII nerve ganglion* has become separated from the *VIII*, and has given rise to the *hyoid* and *mandibular* branches. The *IX ganglion* has sent a nerve into the third arch. The *X ganglion* has divided into the *ganglion jugulare* and *ganglion nodosum*, and the latter is giving rise to the *vagus nerve*.

The Cranial Motor Nerves. — The *III nerve* has entered the *ciliary ganglion*, and the *VI nerve* has just appeared. The *XII nerve* has also begun to develop.

VI. THE ORGANS OF SPECIAL SENSE

The Eye. — Pigment is presented in the *outer wall* of the *optic cup*. On the *inner wall* the *internal limiting membrane* is developing and beneath this in the region of the fundus, axones of the *retinal neuroblasts* are growing into the optic stalk. The *choroid fissure* has partly closed, and its proximal end is filled with the ingrowing *pecten*. The inner wall of the *lens* is continuing to thicken. The middle layer of the *cornea* has begun to develop.

The Ear. — There is no characteristic change directly connected with the ear at this time. Within the pharynx, however, the formation of the *tubo-tympanic cavities* has begun.

The Olfactory Organs. — The depression of the *pits* has greatly increased, and their openings now lie on the border of the oral cavity. The *olfactory epithelium* is giving rise to the elements of the *I nerve*.

Besides describing the events of the fourth day, this chapter also includes an account of the subsequent development of the nervous system and the organs of special sense.

VII. THE URINOGENITAL SYSTEM

The Excretory System. — *Primary tubules* have developed throughout most of the *mesonephros*, while *secondary* and *tertiary tubules* are arising. *Collecting tubules* are springing from the *Wolffian duct* to connect with the two latter types. The *Malpighian bodies* are beginning to appear in the functional portion of the organ which starts to act as a kidney at this time. Rudiments of the *metanephros* are evident as a diverticulum from the posterior end of each *mesonephric duct*. The *nephrotomal tissue* just behind the *mesonephros* is beginning to degenerate.

The Genital System. — The *Gonads* are represented by thickenings of the peritoneal epithelium on either side of the dorsal mesentery, and contain *primordial germ cells*. The *oviducts* are present in both sexes in the form of the *tubal ridges*.

VIII. THE ADRENALS

The *cortical substance* of the adrenal bodies appears on the peritoneal wall near the mesonephros, and material from the primary sympathetic nervous system which is to form the *medullary substance* comes in contact with it.

IX. THE AMNION AND ALLANTOIS

The *amnion* is completed upon the fourth day, while the *allantois* has pushed out somewhat further into the extra-embryonic coelom.

T

HE CHICK: DEVELOPMENT DURING THE FIFTH AND SUBSEQUENT DAYS

THE EXTERNAL APPEARANCE

GENERAL

DURING the fifth day, the cervical flexure reaches its maximum curvature and from then on becomes less and less marked, while the protuberance caused by the mid-brain also attains its greatest relative prominence at this time. The third and last visceral cleft closes during the fifth day, and the future neck is slightly indicated; the first three visceral arches, however, are still somewhat in evidence in this region. The limb buds which were merely rounded swellings on the fourth day are beginning to give evidence of joints.

By the seventh day the second and third arches are no longer visible externally, the heart has moved backward so that the neck is clearly defined, and the external auditory meatus has appeared, as indicated in the previous chapter. The limbs are distinctly jointed, and by the eighth day, the fore limbs begin to appear winglike. Upon the eighth day feather germs are also visible, the tail is relatively much shorter, and the position of the abdominal viscera is quite clearly marked by an external protrusion. From this time on, the embryo gradually assumes a typical birdlike form, one of the most striking changes being the relative increase in the size of the body as compared with that of the head due to mitosis and rearrangement of cells (Gaertner, '49) (Fig. 222).

THE FACE

In connection with the development of the nose and mouth, the face undergoes so great a change between the fourth and eighth days, that it seems best to treat the subject separately.

At four days the openings of the olfactory pits are separated by a median projection overhanging the mouth. It is the *naso-frontal process*. Dorso-laterally each pit is further bounded by the *lateral nasal process* lying between the pit and the antero-dorsal part of the eye. Just below each lateral process there is also another slight out-pushing adjacent to

the antero-ventral side of the eye, termed the *maxillary process* (Fig. 223). During the fifth day the lateral nasal process of either side becomes more closely united with the maxillary process beneath it, the two being separated only by the shallow *lachrymal groove*. At the same



Fig. 222.—Embryo of 7 days' and 7 hours' incubation x5. From Lillie (*Development of the Chick*). After Keibel and Abraham.

time an extension of these united processes crosses each nasal pit and fuses with the frontal process, thus dividing the pits into antero-dorsal and postero-ventral halves. Thereafter as development proceeds the former are carried forward as the *external nares* while the latter are drawn back within the mouth as the *internal nares* (Fig. 224). It is thus evident that the middle portion of the upper jaw is to be derived from the nasofrontal process, and the lateral parts chiefly from the maxillary process. The lower jaw is molded upon the ventral and main part of the mandibular arches (see below). By virtue of these changes the eighth day finds the nares and rudimentary

beak quite clearly defined, the latter being developed by the cornification of epidermal cells about the margins of the jaws. Further growth of these parts, accompanied by a relative diminution in the size of the eye and the development of the eyelids, brings the face to the condition found at the time of hatching.

FEATHERS

In a preceding paragraph feather germs were mentioned, and because of the peculiarly characteristic nature of these structures in the whole class of Birds, it seems desirable to indicate very briefly the essentials of their development.

Feathers, like hair, which we shall consider briefly in connection with the Mammal, are epidermal structures. That is to say, the feather consists of hardened tightly packed epidermal (ectodermal) cells, not of a secretion by cells. Initially a point on the skin where the feather is to appear develops a slight depression, in the midst of which rises a small upgrowth or *papilla*. The apex of the papilla at first is at about the level of the rim of the surrounding depression, or slightly above it. It consists of a core of dermis (mesoderm) covered by the

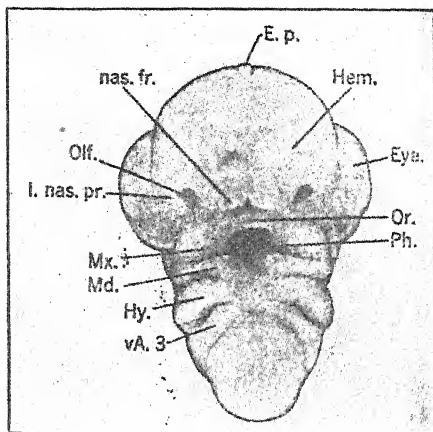


Fig. 223. — Head of an embryo of 4 days' incubation, from the oral surface (N.L. 6 mm.). From Lillie (*Development of the Chick*).

Ep. Epiphysis. *Hem.* Cerebral hemisphere. *Hy.* Hyoid arch. *l.nas.pr.* Lateral nasal process. *Md.* Mandibular arch. *Mx.* Maxillary process. *nas.fr.* Naso-frontal process. *Olf.* Olfactory pit. *Or.* Oral cavity. *Ph.* Pharynx. *vA.3.* Third visceral arch.

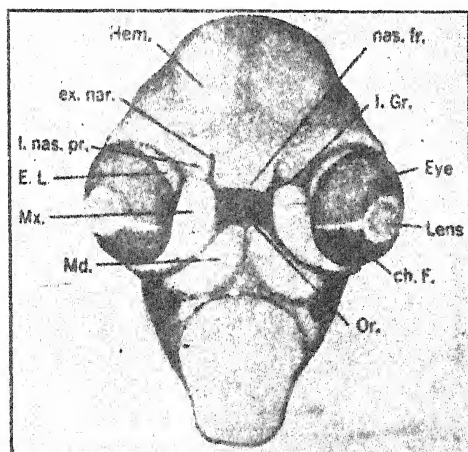


Fig. 224. — Head of an embryo of about 5 days from the oral surface. (N.L. 8 mm.) From Lillie (*Development of the Chick*).

ch.F. Choroid fissure. *E.L.* Eye-lid (nictitating membrane). *ex.nar.* External nares. *l.Gr.* Lachrymal groove. Other abbreviations as in Fig. 223.

usual Malpighian layer of the epidermis, and a thin layer of stratified and cornified epithelium cells, the corneum. In other words it possesses the same cell layers which constitute the skin in other regions.

Very shortly this papilla grows outward so that it protrudes definitely above the level of the rim of the depression, at which stage it is known as a *feather germ*. Within this germ the vascular dermal core is now known as the *feather pulp*. At

the same time the Malpighian layer of the epidermis at the distal end of the germ forms folds whose cells are modified to make the *barbs*. More proximally the folds arise from a nonfolded part of the Malpighian layer whose cells produce a single axis, the *quill*. The latter structure pushes upward and soon throws off its sheath of coreum, emerging as a *down feather*, i.e., a short quill with many short, soft barbs. At the base of the down feather the dermis produces the *pulp* of the permanent feather, while the Malpighian layer here forms two main folds opposite each other, the *rachis*, other lesser folds again producing the barbs. It is interesting to note that transplantation experiments by Cairns, '54, have shown that the underlying dermis determines the special type of epidermal structure which will be formed, i.e., wing feather, leg feather, claw, or scale.

THE SKELETON

As in the case of the Frog, only a brief description of the development of the skeletal system will be given. For a more extended study, the reader is referred to Lillie's *Development of the Chick*, and the books of reference cited therein.

THE VERTEBRAE, THE RIBS, AND THE STERNUM

At the end of the fourth day the cephalic portion of each sclerotome was beginning to fuse with the caudal portion of the one anterior to it to form the rudiment of the right or left half of a vertebra. The occurrence of these vertebral rudiments thus necessarily alternated with the myotomes. An extension of mesenchyme had also grown up on either side of the nerve cord above both the cephalic and the caudal divisions of every sclerotome, forming in each case the respective posterior and anterior rudiment of a future neural arch. This reversed cephalic and caudal relationship between the original sclerotome on the one hand, and the future vertebrae and their arches on the other, is of course a corollary to the alternative arrangement between the vertebrae and myotomes just indicated.

Upon the fifth day, the fusion of the cephalic portion of each sclerotome with the caudal portion of the next anterior to it is completed. The sclerotomes upon one side of the notochord also have become fused above and beneath it with the corresponding sclerotomes upon the other. Furthermore, as a result of concentration, all of the sclerotomal tissue is beginning to become membranous, and in the region of each future vertebra certain portions of this membrane appear especially condensed.

FIFTH DAY: VERTEBRAE, RIBS, STERNUM 437

One such condensation surrounds the notochord as a ring, constituting the rudiment of a vertebral *centrum*. Another occurs in each of the up-growing primordia of the neural arches, and still another arises in the membranous mesenchyme extending outward between the myotomes on either side of the notochord. Each of the latter extensions represents a *transverse* or *costal process*.

During the sixth to the eighth days these costal processes develop further, and in the thoracic region give rise to the membranous primordia of the dorsal two thirds of the upper parts of the *true ribs*, i.e.,

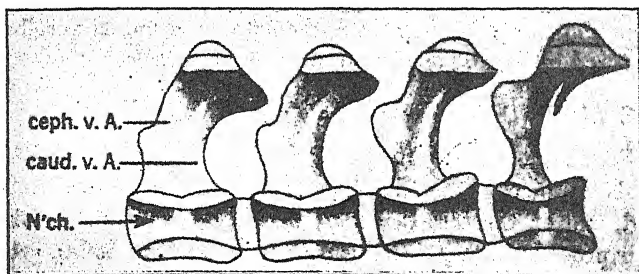


Fig. 225. — The right side of four bisected vertebrae of the trunk of an 8-day Chick. From Lillie (*Development of the Chick*). After Schauinsland.

caud.v.A. Caudal division of vertebral arch. cep.v.A. Cephalic division of vertebral arch. N'ch. Notochord.

those movably articulated to the vertebrae. The cervical costal processes which are not movably articulated are often called *cervical ribs*.¹ The first true rib primordia are those of the fifteenth vertebra, which are followed by six other pairs. The third to the seventh pair of these ribs possess ventral parts which develop from separate centers, and like the ventral one third of the dorsal parts come from lateral plate mesoderm, not sclerotome (Straus and Rawls, '53). The third to the sixth of these parts later fuse to the sternum. Further ventrally, the sternum itself develops from bilateral membranous plates also arising within the lateral plate mesoderm. Presently the membrane of the neural arch primordia unites above the nerve cord, and their normal development seems to be conditioned by both nerve cord and notochord (Waterson, '54). Cartilage formation now starts in all of the regions indicated, and in the last five pairs of ribs the dorsal and ventral part of each has its own center of chondrification. The sternum or breast bone of the chick, including

¹ Since there is no clear cut distinction between cervical and thoracic vertebrae in the Bird, the writer is arbitrarily defining as thoracic all vertebrae with freely articulating or true ribs.

heel likewise has two cartilage forming centers, one in each of the lateral membranous plates; these, however, soon fuse. Following chondrification the cartilage is in turn replaced by actual bone; during this procedure the remains of the notochord are completely eliminated. Such ossification is well advanced by the sixteenth day.

Subsequent to this time several of the thoracic and lumbar vertebrae become rather firmly united with one another, and these in turn are fused to the coalesced vertebrae of the sacral region. To this mass there is also added posteriorly a number of the caudal vertebrae, so that a considerable portion of the spinal column is virtually inflexible, a condition peculiar to the Birds. Lastly, the extreme terminal vertebrae are likewise fused into a single piece termed the *pygostyle*.

THE APPENDICULAR SKELETON

The Fore-limb. — On the fourth day a concentrated mesenchymal mass—probably of sclerotomal origin appears in the base of each fore-limb bud, and on the fifth day there grow out from this membranous mass four processes. One, the *primordium of the limb bones*, grows out into the lengthening wing bud; a second, the *scapula*, grows backward and dorsally above the ribs; a third, the *coracoid*, grows down posteriorly toward the region of the sternum; and a fourth, the *clavicle*, grows in front of the coracoid toward the median line. The last three elements represent the rudiments of the *pectoral girdle*. Centers of chondrification occur in the membranous primordia of the scapula and coracoid on the sixth day, followed later by ossification. The clavicle, on the other hand, ossifies directly from membrane, about the eighth day. Like the coracoid and scapula, all the bones of the fore-limb pass through both a membranous and cartilaginous stage previous to ossification. It is interesting to note that in the wrist there are 13 membranous elements which as a result of fusions produce only two definitive *carpals*. Likewise in the hand five digits are represented in the membrane, but the first and fifth soon disappear.

The Hind-limb. — Like the fore-limb, the parts of the *pelvic girdle* and hind-limb bones arise about the fifth day as four processes from a common mass of mesenchyme in the region of each hind-limb bud. The membranous process representing the limb bones grows out into the bud; another process, the *ilium*, which is elongated in an anterior posterior direction, grows dorsally; a third, the *pubis*, grows antero-ventrally, and a fourth, the *ischium*, grows postero-ventrally. By the

eighth day, the distal ends of the pubis and ischium have both rotated posteriorly so that they are parallel with one another, and with the ilium. Chondrification and ossification follow the membranous stage, and the limb develops in a manner fundamentally similar to that of the fore-limb. There are three *tarsal* elements and five *digits* present in cartilage, but the rudiment of the fifth digit soon disappears. Later the two proximal tarsals fuse with the *tibia*, and the distal one with the three long *metatarsals*; subsequent to ossification the latter become united, thus forming with the distal tarsal element the single *tarso-metatarsus*.

As regards the details of ossification in the long bones of the Chick, we find that the situation differs somewhat, both from that in the Frog, and from what we shall later see in the Mammal. As noted the membranous stage is as usual followed by cartilage, and as in the Frog in the region of the shaft or diaphysis this cartilage is overlaid by periosteal bone. In this case, however, the cartilage is presently destroyed, and partly replaced by true endochondral bone, though of a cancellous character. Throughout the shaft this cancellous endochondral bone is then likewise removed to be replaced to a considerable extent by marrow. Thus in respect to having most of each long bone ultimately of periosteal and membranous origin the Bird approaches, but does not quite equal the condition in the Frog. There is in the Chick some endochondral ossification of a permanent nature in these bones which comes about because of their method of longitudinal growth which takes place as follows:

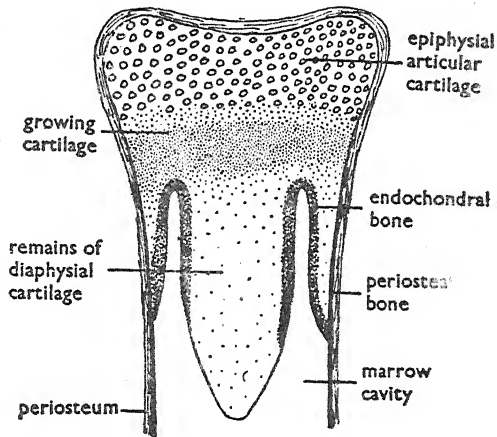


Fig. 226. — The head of a long bone (femur) in the Chick. From Lillie, after Brachet. The situation with respect to the epiphysal cartilage differs from that in the Frog, but the situation in the diaphysis is similar to the extent that, save at the ends, there is little or no bone except that produced by the periosteum.

The epiphyses or ends in the Chick bones, unlike those in the Frog, only remain cartilaginous during the increase in length of the diaphysis.

This increase occurs through ossification of the cartilaginous ends on their diaphyseal sides, with simultaneous addition of more cartilage distally (Fig. 226). Finally as growth is completed the cartilage of the epiphyses is entirely replaced by cancellous bone. In this manner it happens that a little spongy bone at the ends of the diaphysis, and all of that in the completely ossified epiphyses is of endochondral origin. In concluding this topic it should be noted that among the long bones of the Bird the humerus is peculiar in one respect. In this bone there is relatively little marrow, the extensive cavity therein being largely occupied, as will presently be noted, by one of the lung outgrowths called air sacs. (See below.)

THE SKULL

The Primordial Cranium.—The primordial or cartilaginous cranium of the Chick is first indicated by concentrations of mesenchyme during the fourth and fifth days. Then, during the sixth, seventh, and eighth days, these mesenchymal concentrations develop into the following fused elements of cartilage. Along either side of and encasing the anterior end of the notochord, appear the *parachordal plates*. In the Chick these elements develop from the first as a single piece, and are often known, therefore, as the *basilar plate*. Anterior to it are developed simultaneously upon either side another pair of plates—the *trabeculae*. Posteriorly, these are continuous with the parachordals, with which they form an angle corresponding to the cranial flexure, while anteriorly, their ends meet and fuse with one another. This fusion then extends somewhat, so that eventually the central space is closed, except for a small opening containing the pituitary body. Thus the trabeculae and parachordals together form the entire cartilaginous floor of the skull.

At the same time that these plates are forming, cartilage also develops around the auditory sacs and the olfactory organs, forming respectively the *auditory* and *olfactory capsules*. These are in direct continuity eventually with the plates. From the postero-dorsal part of each auditory capsule, processes now grow toward one another and fuse above the hind-brain. Thus is constituted the only portion of the roof of the cranium which is preformed in cartilage. Posterior to each auditory capsule, a dorso-lateral plate of cartilage develops, while anterior to and in contact with the capsule, a transverse partition arises between it and the orbit. This partition extends medially somewhat, so as partially to bound the brain cavity in front. Anterior to the cranial cavity, mid-

way between the two orbits, and between the nasal capsules, a continuous longitudinal partition appears and fuses ventrally with the trabeculae. It is the *interorbital* and *internasal septum*.

The remaining part of the skull which is preformed in cartilage is known as the *visceral skeleton* or *cartilaginous splanchnocranium*, and arises from the first three pairs of visceral arches. During the fifth day, these arches are chiefly membranous, and the antero-ventral or distal ends of the first mandibular pair have fused with one another in the middle line. Subsequent to the fifth day, the ventral or main parts of each mandibular arch become chondrified, and are known as *Meckel's cartilages*; they form the core of each side of the lower jaw. From the proximal (i.e., hinder and upper) end of each of these arches, there develops a tri-radiate piece of cartilage, the *palato-quadrate*, which eventually ossifies as a separate bone. It is termed simply the *quadrate*, and constitutes the articulation between the lower and upper jaws. The second (hyoid) and third visceral pairs of arches later form the hyoid apparatus, consisting respectively of the paired *lesser* and *greater cornuae* and the two median *copulae*. Moreover, the upper ends of the second arches are thought to give rise to parts of the columellae, as noted in the account of the ear (Chapter 12).

Altogether, the final bones of the Bird's skull which have been preformed in cartilage are the following: the *basi-occipital*, *exoccipitals*, and *supra-occipitals* about the foramen magnum; the *proötic*, *epiotic*, and *opisthotic* about each auditory capsule; the *basisphenoid*, *orbitosphenoids*, *alisphenoids*, and *interorbital* and *internasal septum* about the eyes and nasal capsules; the *quadrate*, and *Meckel's cartilages* in connection with the lower jaw; and the *hyoid apparatus* in the region of the throat.

The Membrane Bones. — These are bones which are not preformed in cartilage, but ossify directly from the condensed mesenchyme or membrane. They constitute a good share of the Bird's skull, and begin to develop about the ninth day. The bones thus formed are as follows: the *parietals*, *frontals*, and *squamosals*, forming together the main part of the cranium proper; the *lachrymals*, *nasals*, and *premaxillae*, forming the face and part of the upper jaw; the *maxillae*, *jugals*, *quadratojugals*, *pterygoids*, *palatines*, *parasphenoids*, and *vomer*, forming the rest of the upper jaw and the base of the cranium; and the *angulars*, *supra-angulars*, *operculars*, and *dentals*, forming the covering bones for the lower jaw.

THE ALIMENTARY TRACT

THE FORE-GUT REGION

The development of the mouth proper has already been sufficiently described in connection with the discussions of the alimentary tract and the middle ear in Chapter 12, and of the skull in the preceding paragraph. We shall proceed, therefore, to an account of the further development of the remainder of this tract and its appendages.

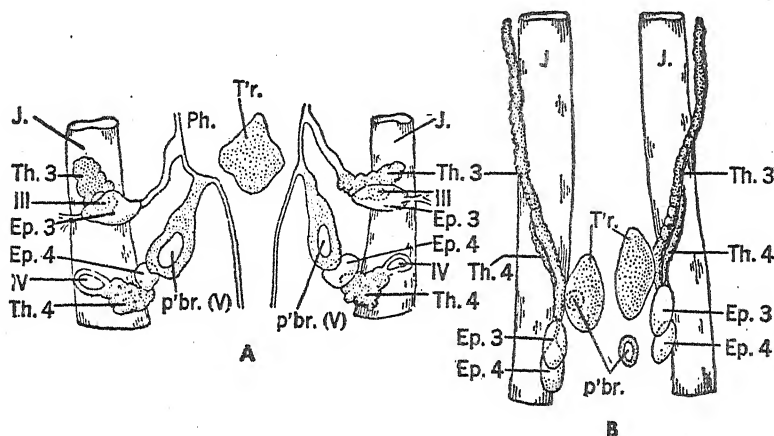


Fig. 227. — Derivatives of the visceral pouches and associated organs, in the Chick. From Lillie (*Development of the Chick*). After Verdun (Maurer). Combined from frontal sections. A. In embryo of 7 days. B. In embryo of 8 days.

Ep. 3, Ep. 4. Epithelial vestiges derived from ventral portions of the third and fourth visceral pouches. *J.* Jugular vein. *p'br., p'br. (V).* Postbranchial bodies derived from fifth visceral pouch. *Ph.* Pharynx. *Th. 3, Th. 4.* Thymus bodies derived from dorsal portions of the third and fourth visceral pouches. *T'r.* Thyroid body. *III, IV.* Remains of third visceral cleft and position of fourth which never becomes a real cleft.

The Visceral Pouches and Arches.

The Pouches. — At the end of the fourth day, the first and second visceral clefts had closed, and the third had opened; during the fifth day, this latter cleft also closes, whereas the fourth pouch, it will be recalled, has never developed an outer opening. About the seventh or eighth day, the third and fourth pouches sever their connections with the pharynx, and thus remain as patches of epithelium in the mesenchyme of the neck, adjacent to the jugular vein. The dorsal portion of the epithelium from the third pouch then fuses with the dorsal portion from the fourth to form a *thymus* body on each side of the throat of the

young Chick. Though thus apparently endodermal, Hammond, '54, states that the clefts rather than the pouches may be the source of the thymus and hence that it is ectodermal. Epithelial vestiges of the third and fourth pouches are generally thought to produce the *parathyroids*, while each fourth pouch also produces a posterior outpushing sometimes regarded as a vestigial fifth pouch. These separate from the pouches, and the left one becomes the *post-branchial body*, somewhat like a small parathyroid, while the right one degenerates (Fig. 227). Dudley, '42, thinks these outpushings may be rudimentary sixth pouches, the fifth having fused with the fourth.

The Arches. — The fate of the first three pairs of visceral arches has already been sufficiently described above in connection with the visceral chondrocranium. The fourth pair of arches never develop beyond a mesenchymal state and eventually disappear. The fifth pair are vestigial and even more transitory.

The Respiratory Tract. — At the end of the fourth day, the respiratory tract consisted of the glottis, the larynx, the trachea, and a pair of posterior outgrowths from the latter, the rudiments of the bronchi and lungs. All these parts, having arisen from the fore-gut, are necessarily lined by endoderm. Upon the fifth day, however, the mesenchyme about them begins to condense to form true mesoderm, through which the lung rudiments continue to grow posteriorly as a pair of tubes. Upon the sixth day, these tubes begin to branch, and thus it appears that the original rudiments really represented the lining of only the two main or primary bronchi. Their branches then constitute the linings of the *secondary bronchi*, and the intercommunicating *tertiary* or *parabronchi*, together with the finer ramifications from the latter known as *air capillaries*. This network of air capillaries, it is to be noted, takes the place of the blind terminal sacs or alveoli found in the Mammals. Thus there are no pockets of residual air in the lungs of the Bird, but continuous passages which make possible a complete circulation. The mesoderm indicated above eventually gives rise in the region of the larynx and trachea to the cartilages and muscles of these organs. Further back it surrounds the endodermal lining of the various bronchi and air capillaries, and ultimately forms the connective tissue substance of the lung. Through this tissue the blood vessels later ramify among the tubes and capillaries.

In the case of the Bird, besides these tubes and respiratory capillaries, there are also connected with the lungs the various *air sacs*. These arise, with one exception, as outgrowths from the secondary bronchi, the exceptional case being the abdominal sacs which originate directly

from the posterior ends of the primary bronchi. The rudiments of the abdominal and cervical sacs are said by some to be distinguishable as early as the fifth day, while the others appear somewhat later (Fig. 228). In the course of development these peculiar sacs which have thus originated, gradually push their way to their respective positions among

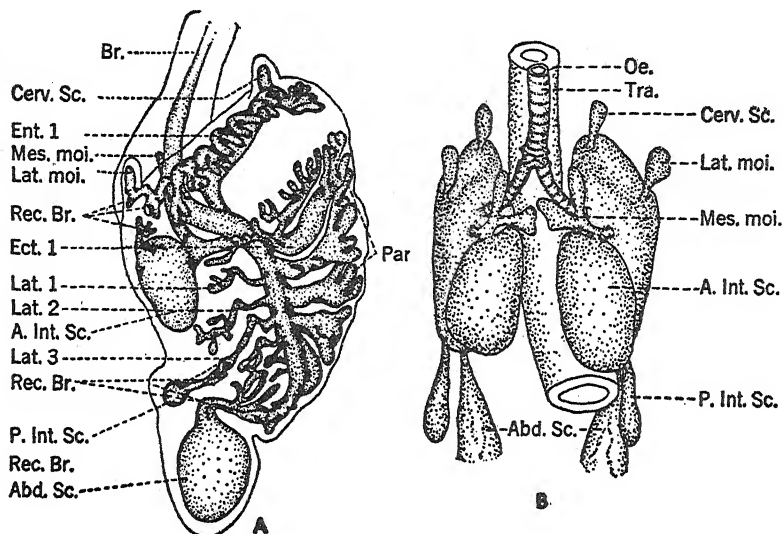


Fig. 228. — *A.* Lateral view of the left lung of a 9-day embryo, showing branches of the bronchi within it. *B.* Ventral view of the lungs and air-sacs of a 12-day embryo, with internal branches of the bronchi not shown. After Locy and Larsell.

Abd. Sc. Abdominal air-sac. *A. Int. Sc.* Anterior Intermediate air-sac. *Br.* Extra-pulmonary bronchus. *Cerv. Sc.* Cervical air-sac. *Ect. 1.* An ectobronchus. *Ent. 1.* An entobronchus. *Lat. 1, 2, 3.* Laterobronchi. The ecto. ento. and laterobronchi are all classed as secondary bronchi in the text description. *Lat. moi., Mes. moi.* Lateral and mesial moieties of interclavicular air-sac. *Oe.* Oesophagus. *Par.* Parabronchi. *P. Int. Sc.* Posterior Intermediate air-sac. *Rec. Br.* Recurrent bronchi.

the viscera. Here they come to occupy considerable space, while a branch of the interclavicular sac extends eventually even into the upper bone (humerus) of each wing.² Besides being connected with the respiratory passages by the bronchi from which they arose, each sac, with the exception of the cervicals, also develops secondary connections with the parabronchi. In the adult these connections always convey air from the

² In the latter case the bone is said to undergo a kind of dissolution to make way for the ingrowing sac, and the dissolution is thought to depend on parathyroid activity which in turn is due to oestrogens derived from the yolk-sac (Bremer, '40).

sacs to the lungs, and are, therefore, termed *recurrent bronchi*. The cervical sacs, though possessing no recurrent bronchi, are indirectly connected with branches of the most anterior pair of secondary bronchi, and these branches probably act as recurrences. The functions of the sacs are apparently to lighten the Bird's body, to help maintain air currents and, in the case of the abdominal sacs, to cool the testes.

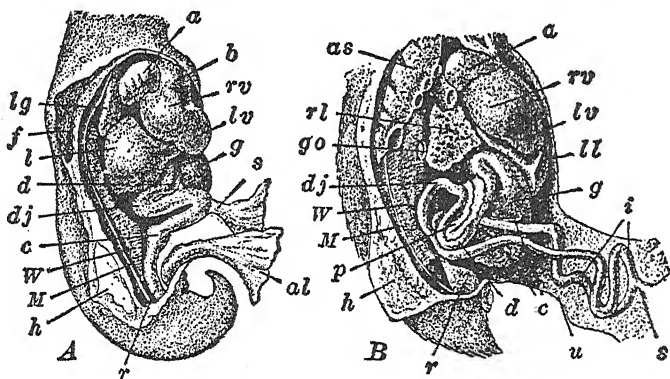


Fig. 229. — Partially dissected viscera of the Chick, from the right side. From Kellicott (*Chordate Development*). After Duval. A. Of a 6-day Chick, enlarged slightly less than six times. B. of a 13-day Chick, enlarged two and one half times, showing the elongated intestine and its extension into the umbilical stalk.

a. Right atrium. al. Allantois. as. Abdominal air-sac. b. Bulbus arteriosus. c. Caecal processes. d. Loop of duodenum. dj. Duodenal-jejunal flexure (a relatively fixed point during the elongation of the intestine). f. Fore-limb bud (cut through). g. Gizzard. go. Gonad. h. Hind-limb bud (cut through). i. Loops of small intestine. l. Liver. lg. Lung. ll. Left lobe of liver. lv. Left ventricle. M. Rudiment of Mullerian duct (tubal ridge). p. Pancreas. r. Rectum. rl. Right lobe of liver. rv. Right ventricle. s. Yolk-stalk. U. Umbilical stalk. W. Wolfian body or mesonephros.

Finally, in connection with the development of the respiratory system, it is to be noted that about the fifth day, the glottis begins to close. Both larynx and glottis later become entirely shut, but subsequent to the eleventh day, the opening is gradually re-established.

The Esophagus, the Stomach, and the Duodenum. — At the end of the fourth day, the esophagus was a straight tube, while the region of the stomach and duodenum was indicated by a slight curvature to the left. The esophagus does not alter much on the fifth day, except to continue to elongate. The stomach, however, is becoming distinguished from the duodenum by its greater dilation. Also, at the extreme left of the gastric duodenal curve, a slight pouch is forming. This

marks the end of the gastric region. Later this pouch enlarges to form the muscular *gizzard*, while the part between it and the esophagus develops the gastric glands and comprises the *proventriculus*. The *crop* is evident by the eighth day as a dilation of the esophagus at the base of the neck. Anterior to the crop at that time, the lumen of the esophagus is temporarily closed.

The *duodenum* is not very clearly defined on the fifth day, but shortly afterward it begins to develop as a loop in the tract just beyond the gizzard. From the gizzard, the proximal limb of the loop descends a short distance, and then bends upward to form the ascending branch. Ultimately the pancreas comes to lie in between the limbs of this loop. The end of the ascending branch marks the termination of the original fore-gut region and the beginning of the small intestine (Fig. 229).

The Liver.—On the fifth and subsequent days, as on the fourth day, development of the liver consists chiefly in further growth in size. This is accomplished as already indicated by continuous branching and anastomosing of the original diverticula together with the accompanying blood capillaries. These diverticular branches are at first solid, but on the fifth day many of them have acquired a lumen, and this process continues as growth proceeds.

As regards the bile ducts, it is to be noted that on the sixth day the common duct disappears, and the two bile ducts which emptied into it again empty directly into the duodenum.

The Pancreas.—The pancreas at four days, it will be recalled, consisted of three separate outgrowths: a dorsal one from the wall of the duodenum opposite the common bile duct, and the beginnings of two ventral ones from the duct itself. During the fifth day all three diverticula continue to grow and branch (Fig. 230). On the sixth day, the right ventral pancreatic mass becomes united with the dorsal, whose duct shifts ventrally on to the left side of the duodenum. As noted above, the common bile duct disappears at this time, and thus the two ventral pancreatic ducts come to open directly into the intestine. Later, the left pancreas becomes fused with the other two, and there remains a single glandular mass lying in the mesentery within the loop of the duodenum. Its three ducts continue to remain separate, however, and they open into the distal limb of the duodenal loop near the bile ducts.

THE MID-GUT REGION

It has been indicated that the mid-gut or rudimentary small intestine begins at the end of the duodenum. At the close of the fourth day, it

was noted that it extended from this point as a virtually straight tube across the region of the umbilicus to the beginning of the tail fold and hind-gut. In about the middle, it gave off the yolk-stalk. During the fifth day a very slight downward bend (the *duodeno-jejunal flexure*)

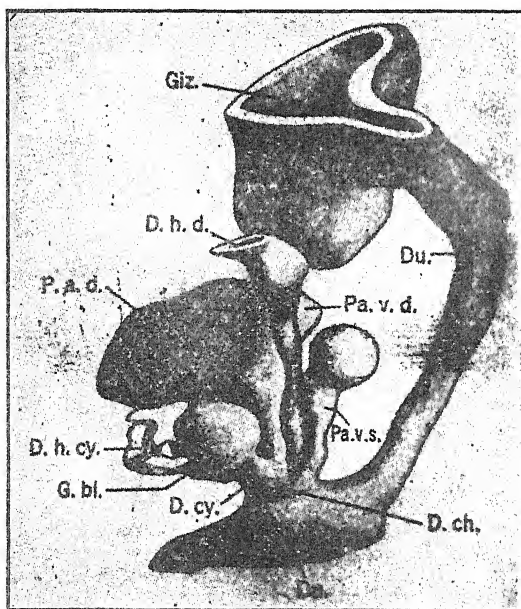


Fig. 230. — Reconstruction of gizzard, duodenum, and hepato-pancreatic ducts of a Chick embryo of 124 hours. From Lillie (*Development of the Chick*). After Brouha.

D.ch. Ductus choledochus. *D.cy.* Ductus cysticus. *D.h.cy.* Ductus hepato-cysticus. *D.h.d.* Dorsal or hepato-enteric duct. *Du.* Duodenum. *G.bl.* Gall bladder. *Giz.* Gizzard. *Pa.d.* Dorsal pancreas. *Pa.v.d.* Right ventral pancreas. *Pa.v.s.* Left ventral pancreas.

appears just at the point where the duodenum ends and the mid-gut begins. From this bend, the latter extends postero-ventrally for about half its length; at this point, as noted, it connects with the yolk-stalk. It then ascends again to its termination, which is now marked by a small bilateral swelling, the rudiment of the *intestinal caeca*. The entire mid-gut region thus indicated is still quite short, and its dip down into the umbilical stalk very slight.

On the sixth day, however, the ventral dip of the small intestine reaches well down into the above stalk, thus forming in the intestine

as a whole a second distinct loop (Fig. 229, *A*). The latter soon becomes much more pronounced than the duodenal loop, and during later development acquires numerous convolutions (Fig. 229, *B*). These convolutions lie within the umbilical stalk until about the eighteenth day and are then drawn into the body. They are soon followed by the

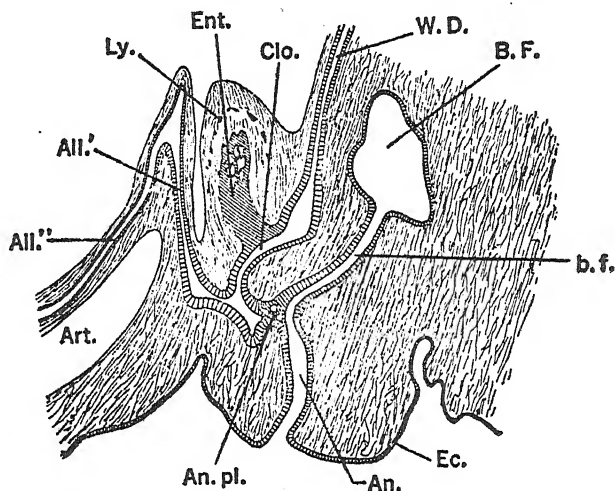


Fig. 231.—Chick embryo of 11 days, sagittal section through the region of the cloaca. Reconstructed from several sections. (After Minot.) From Lillie (*Development of the Chick*). Anterior end toward the reader's left.

All'. Ascending limb of the allantois. *All''*. Descending limb of the allantois. *An*. Anal invagination, or proctodaeum. *An.pl*. Anal plate or cloacal membrane. *Art*. Umbilical artery. *B.F.* Bursa Fabricii. *b.f.* Duct of the bursa. *Clo.* Cloaca, i.e., the urodaeal portion. *Ec.* Ectoderm. *Ent.* Entoderm of the rectum. *Ly.* Nodules of crowded cells, probably primordia of lymphoid structures in the wall of the large intestine. *W.D.* Wolffian duct.

remains of the yolk-sac. The intestinal caeca which were barely indicated on the fifth day ultimately grow out into two fingerlike processes.

THE HIND-GUT REGION

On the fifth day, as on the fourth, there is no particular change in the rectum. On the seventh and eighth days, however, its cavity becomes occluded. Later, the lumen is restored except for a small plug separating it from the cloaca, and just anterior to this plug a slight dilation develops. This dilation is the *coprodaeum*. The plug persists until about the time of hatching.

The chief change in the cloaca during the fifth day is the fusion of

the laterally compressed walls of the posterior part. During subsequent development, a cavity is re-established in the postero-dorsal part of this closed portion; it constitutes the *bursa Fabricii* of the adult. This is a sac which remains separate from the original cloaca, but which opens into another cavity, communicating directly with the exterior. This

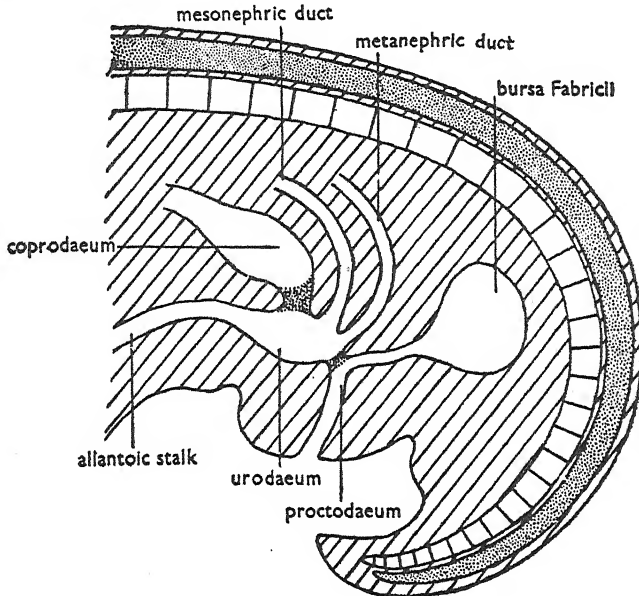


Fig. 232. — A diagram of a sagittal section of the posterior end of an approximately eleven-day embryo to indicate better the relations of the parts partially shown in Fig. 231. The metanephric duct opening separately into the urodaeum (a condition attained on the sixth day) is shown, though for some reason it does not appear in Fig. 231. The anal plate separating urodaeum from proctodaeum is shown in the diagram, but is unlabelled.

latter cavity is the *proctodaeum*, and has arisen by an outpushing of the ectodermal walls around the edges of the anal plate or cloacal membrane (Figs. 231, 232; compare Fig. 193, Chapter 10). At hatching the latter disappears and thus the proctodaeum is finally placed in communication with the original embryonic cloaca minus the posterior portion of the latter which went to form the *bursa Fabricii*. At about the same time the plug which closes the rectum disappears. Thus, the adult cloaca consists of three parts, the coprodaeum, a part of the original cloacal chamber now called the *urodaeum*, and the proctodaeum. The latter opens to the outside through the *anus*.

THE CIRCULATORY SYSTEM

THE HEART

During the fourth day a series of changes in the position of the various parts of the heart in relation to each other were indicated. During the fifth day these changes progress rapidly, and upon the sixth day are virtually completed.

Besides these movements, there were also noticed on the fourth day the beginnings of certain partitions within the heart. These were the interatrial, the interventricular, and the cushion septa. During the fifth and part of the sixth days, all these are practically completed. This process involves, first, the meeting of the two parts of the cushion septum so as entirely to divide the atrio-ventricular canal into right and left channels. The interatrial septum then unites with the cushion septum on the antero-dorsal side of the latter, while the ventricular septum joins it postero-ventrally. These fusions, though described separately, occur more or less simultaneously (Fig. 209, *F*).

In connection with these processes there remain to be added certain details as follows: As the division of the originally single atrium into two atria occurs communication between them is preserved by the concomitant development of perforations in the newly formed septum. These perforations correspond functionally to the *foramen ovale* in the heart of the Mammal, and their physiological significance is described below. It must also be noted that the interatrial septum as thus far described is augmented in the adult Bird by the addition of another part as follows: Upon the seventh day the proximal portions of the left precava and the pulmonary vein start to be incorporated into the atria, and as this occurs the tissue between them is added to the septum. This new part is called the *pars cavo-pulmonalis* (Quirring, '33). Lastly, there is also a small ventricular foramen whose final closure will be described presently in connection with the development of the aortic division of the bulb.

This completes the description of the septa within the heart proper. Upon the fifth day, however, another septum develops within the truncus arteriosus. It appears first at the anterior end of this vessel in such a position as to separate the orifice leading to the sixth aortic arches and hence to the pulmonary arteries, from that which leads to the third and fourth aortic arches. This partition then grows backward through the

distal portion of the bulbus, and on the sixth and seventh days it connects with a septum which has formed within the proximal portion of that vessel. Thus a continuous somewhat spirally twisted partition has been produced extending through the truncus and bulbus clear in to the interventricular septum of the heart. It is to be noted that the entire bulbus, though now ventral, still lies somewhat to the right of this latter septum. Nevertheless, the fusion of the bulbus septum and interventricular septum is effected in such a way that in connection with subsequent changes in the cushion septum the aortic division (i.e., the division from the third and fourth arches) of the bulbus comes to open through the foramen in the ventricular septum directly into the left ventricle. The pulmonary division, on the other hand, continues to open into the right ventricle (Fig. 233).

Subsequent to the fifth day also, certain other changes are completed as follows. The *semilunar valves* develop in both the aortic and pulmonary divisions of the bulbus, and the parts of that vessel proximal to these valves are incorporated into the ventri-

cles. The two divisions of the bulbus and truncus arteriosus distal to this point are gradually separated so as to form distinct vessels, i.e., the proximal portions of the *aortic* and *pulmonary arteries*. As noted in a previous chapter, the sinus venosus becomes a part of the right atrium into which empty all the systemic veins, and finally both atria acquire small *auricular appendages* or *auricles*.

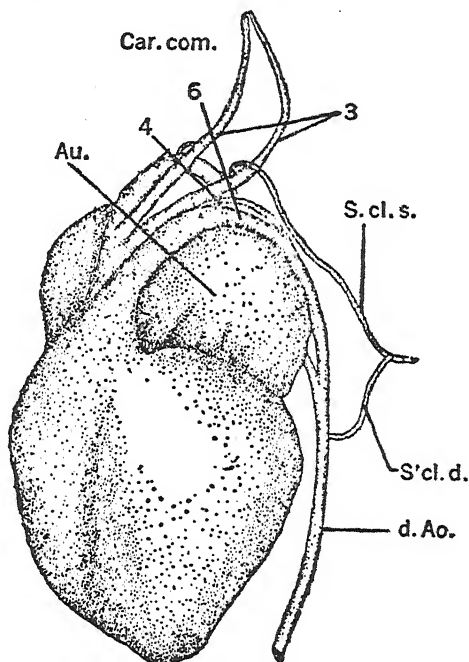


Fig. 233. — The heart and aortic arches of a Chick embryo the latter part of the sixth day. From a dissection. From Lillie (*Development of the Chick*). After Sabin.

Au. Atria. *Car.com.* Common carotid artery. *S'cl.d., S.cl.s.* Primary and secondary subclavian arteries. 3, 4, 6. Third (carotid), fourth (systemic) and sixth (pulmonary) aortic arches.

THE EMBRYONIC BLOOD VESSELS

The Arteries.

The Aortic Arches.—At the end of the fourth day, the pairs of aortic arches which remained fully developed were the third, fourth, and sixth. The third pair, it will be recalled, ran upward from the ventral aorta, and continued anteriorly as the internal carotids, while posteriorly the dorsal end of each of these arches was still connected with the dorsal end of each fourth arch. From the base of each of the third

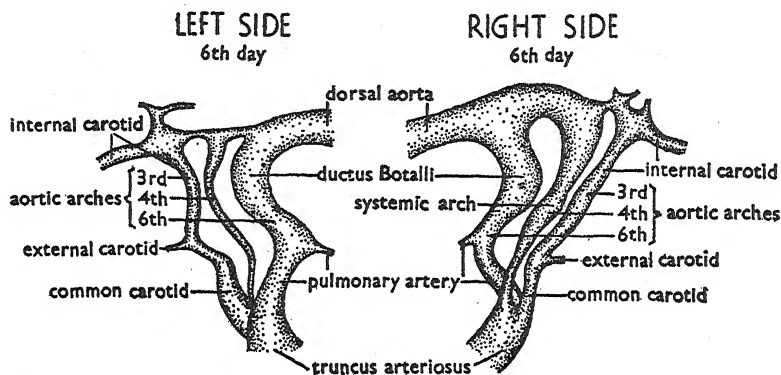


Fig. 234. — Reconstruction of the aortic arches of a 6-day Chick embryo from a series of sagittal sections. Modified from Lillie.

arches, on the other hand, another vessel ran forward as an external carotid.

Upon the fifth day three further changes are initiated as follows. First, on each side, the portion of each dorsal aorta between the third and fourth arches begins to disappear. Secondly, the fourth arch on the left side diminishes in size (Fig. 234). Thirdly, there occurs anteriorly an anastomosis between the internal and external carotids, while the portion of the latter between this point and the base of the third arch (primary external carotid in Fig. 235) begins to atrophy.

By the eighth day the changes thus begun have been completed, so that the condition then obtaining is as follows: First as regards the systemic and pulmonary arches, it is to be noted that on the left side, the entire fourth arch together with the dorsal aorta between the third and the sixth arch has vanished. On the right side the dorsal connection between the third and fourth arches is gone, but the fourth arch itself is well developed. It persists as the *main systemic arch* of the

adult (Fig. 210, *B*). It is to be noted that the Bird differs from the Mammal in that in the latter, it is the left arch which remains. The immediate cause of this interesting difference between Bird and Mammal according to Bremer ('28) is as follows: In the first place in the Bird the torsion of the heart tube is somewhat greater than in the Mammal. Secondly this is correlated with a greater backward movement of the heart in the Bird in connection with the greater length of the neck. This last feature results in lengthening the aortic vessels and in involving them in the increased torsion of the cardiac tube. Thus the left fourth arch is drawn into a disadvantageous position on the ventral side of the truncus, while the right assumes a dorsal position with a much more direct connection with the dorsal aorta (Fig. 236). In the Mammal on the other hand, not only is this not true, but according to Congdon

and Wang ('26) the blood as it comes from the truncus on the right is necessarily directed toward the left. Hence the left arch receives the larger stream and so becomes the dominant vessel.

All parts of the sixth arches continue to be well developed on both sides throughout embryonic life. At the time of hatching, however, the upper portion of each vessel between the origin of the pulmonary arteries and the dorsal aorta (i.e., the duct of Botallo or ductus arteriosus, indicated above) becomes atrophied and remains only as an occasional vestige in the adult.³ In the second place with respect to the carotids it appears that since the atrophy of each external carotid between the base of the respective third arch and the point of its anastomosis

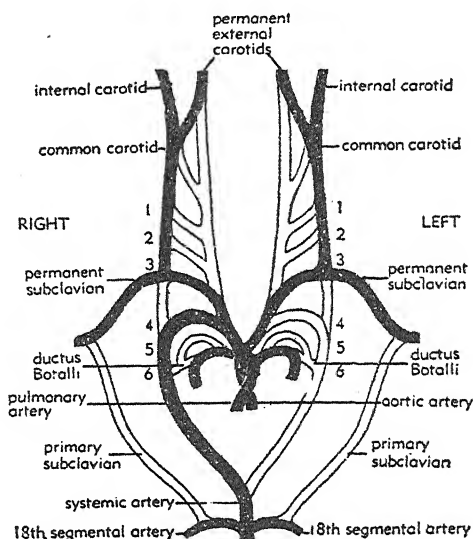


Fig. 235. — Diagram of the aortic arches and connecting vessels in the Chick as viewed from the ventral side. The vessels in outline indicate the situation existing at one time or another in the embryo. Those shown in black indicate the permanent arrangement.

³ In the Mammal a remnant of the left duct of Botallo always persists.

with the internal carotid has been completed, each external and internal vessel now takes its origin and continues anteriorly from this point of fusion. Posterior to this point certain remaining parts constitute on either side a newly named vessel, the *common carotid*. Each common carotid consists of what was previously the postero-dorsal portion of the respective internal carotid, the respective third arch, and the part

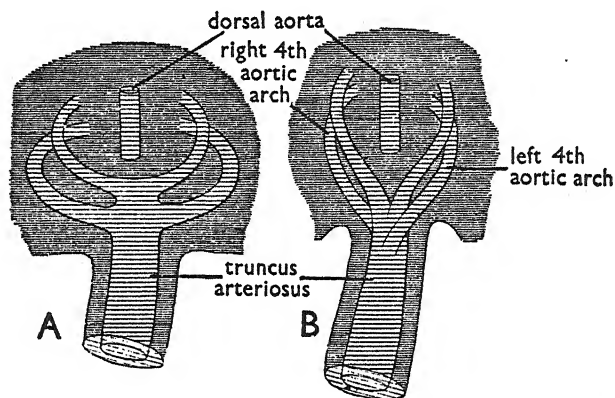


Fig. 236. — Diagrammatic ventral view of the truncus and the third and fourth aortic arches in *A*, the Mammal, and *B*, the Chick. After Bremer. Note that in the Chick the fourth arches are involved in the twist of the truncus, thus bringing the right fourth arch dorsal, and hence nearer to the dorsal aorta. The left fourth arch on the other hand is brought ventrad, and hence further from the dorsal aorta, thus leading to its elimination in this form.

of each ventral aorta proximal to the base of this arch and the point of union with the systemic vessel (Fig. 235). It is to be noted in this connection that the point of anastomosis between each external and internal carotid is not shown in Fig. 234. Hence each vessel there indicated as an internal carotid eventually becomes part of a common carotid. Finally, it must be remembered that while these changes are occurring, the head of the Bird is being separated from the body by the development of the neck. This process results in the backward movement of the heart and all its arches, so that by the time they have reached the stage indicated on the eighth day, they lie entirely within the thorax. The carotids, on the other hand, are elongated into vessels which pass forward into the head.

The Physiological Significance of the Embryological Structure of the Heart and Aortic Arches. — Before considering the remainder of the

blood vessels, it seems well to digress at this time in order to point out the physiological significance of the heart and its arches as they have just been described.

The heart, as has been seen, becomes virtually four chambered. It fails to become entirely so during embryonic life, however, because of the persistence of the foramina in the interatrial septum. This fact, as well as the existence of the dorsal portions of the sixth arches, i.e., the ducts of Botallo, is correlated with the embryonic method of aerating the blood. This becomes clear upon a consideration of what this method involves, as follows:

It is obvious that previous to the hatching of a Bird or birth of a Mammal the lungs cannot act. Instead the allantois of the Bird, or as will later be explained, the partially homologous placenta of the Mammal, performs the function of blood aeration. There now remains to be described the relationship which the interatrial foramina and the ducts of Botallo bear to the distribution of the different classes of blood. The fully aerated blood from the allantois, the nutrient laden blood from the yolk-sac, and a relatively small amount of strictly venous blood from the posterior part of the body become mixed in the ductus venosus, and from thence are poured together into the right auricle. At the same time that this occurs the right auricle is also receiving blood through the ducts of Cuvier or anterior venae cavae (see below). This blood is returning from the head, and hence, save perhaps in the very early stages, is relatively depleted of oxygen and nutriment. Up to this point there is no question about the facts. From here on, however, there have been two distinct theories as to the fate of the two classes of blood just indicated. Both have been developed as a result of observations and experiments upon Mammals, but probably apply equally well in their essential points to Birds.

The first theory was somewhat obscurely outlined by Harvey in connection with his original discussion of the circulation of the blood in 1628. It can be very briefly stated as follows: It holds simply that the two types of blood are completely mixed as they enter the right atrium, and hence that there is no separation of aerated and unaerated blood in the embryo. This has been accounted for on the ground that the organism is sufficiently small and inactive and the circulation sufficiently swift so that such separation is unnecessary. The second theory was developed in 1798 by Sabatier, and may be described thus:

It is supposed that the structure of the right atrium is such that the blood entering it from the posterior part of the body through the ductus

venosus (aerated blood) is turned away from the right ventricle and guided through the aperture or apertures in the interatrial septum into the left atrium. From here it passes into the left ventricle, and thence through the aortic division of the bulbus and truncus arteriosus into the third and fourth aortic arches. The third arches, as has been seen, convey this blood newly oxygenated and full of nutriment straight to the head; the rest passes through the fourth arches (later only one, the right or left) and backward along the dorsal aorta. On its way, however, it becomes mixed with the depleted blood which has returned from the head; this occurs as follows: It was noted above that this blood from the head also passes into the right atrium. According to the present theory, however, its direction of entrance, together with the structure of the cavity, is such that it is diverted from the openings into the left atrium, and emptied directly into the right ventricle. From here it passes out through the pulmonary division of the bulbus and truncus arteriosus, and thence a slight part of it flows through the small pulmonary arteries into the rudimentary lungs. The larger part, however, continues through the dorsal portions of the sixth arches, i.e., the ducts (later only one duct) of Botallo, into the dorsal aortae; here, as indicated above, it inevitably mixes with the aerated blood from the fourth arches (later arch). Some of this mixture then supplies the body posterior to the head. The larger share of it, however, eventually reaches again the walls of either the allantois or the yolk-sac, where it receives respectively oxygen or food material, and is returned to the heart in the manner already noted. Thus the posterior part of the body should get blood poorer in oxygen and nutriment, at least during later stages when the above arrangement would be in operation (Fig. 236X). Hence some think there may be a relation between this and the faster growth of the anterior end, if indeed that end is still growing faster at this time.

However, despite the theoretical considerations in favor of this second theory, all evidence until recently has supported the earlier view. Thus to begin with, in the human embryonic heart near term at least, it was shown anatomically that the interatrial aperture is not large enough to pass all of the blood delivered by the postcaval vein. Hence it would appear that some mixture of blood from the anterior and posterior veins must occur in the right atrium. Then Pohlman, in 1909, apparently settled the matter experimentally by injecting cornstarch into the vessels leading from the placenta of the Pig embryo into the right atrium. He then withdrew equal amounts of blood from each ventricle and found them to contain equal numbers of grains. This type of experiment with certain

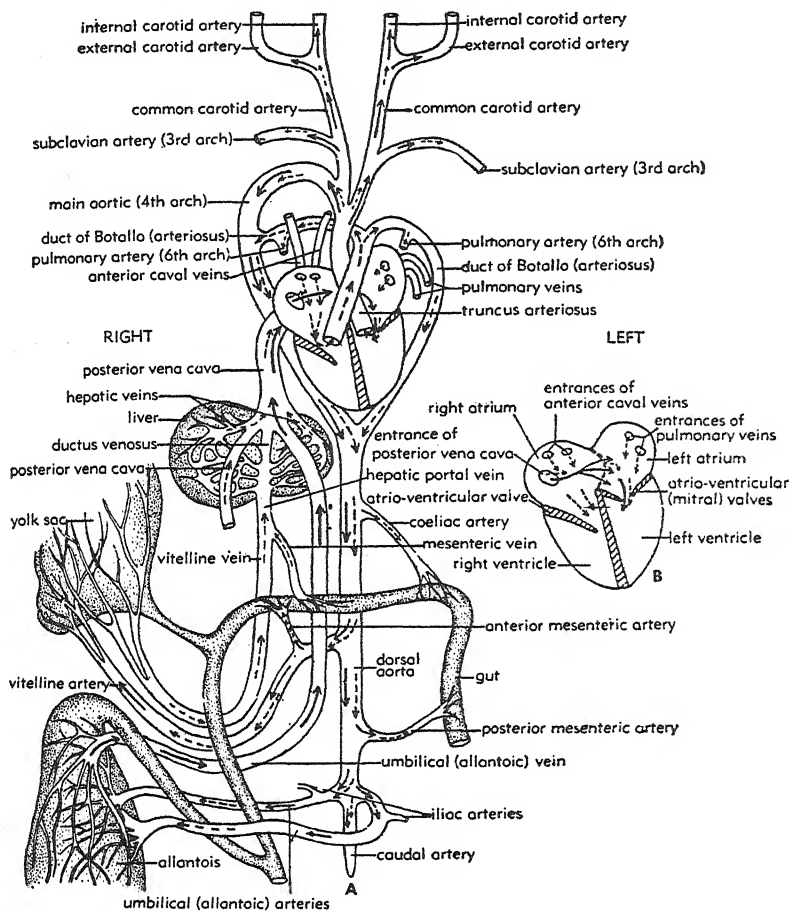


Fig. 236X. — Diagrams to illustrate the circulation in the Chick embryo according to Lillie, and indicating at least a partial separation of aerated from unaerated blood. Solid arrows represent aerated blood and broken arrows unaerated blood, the relative amounts of each type being suggested by the size and heaviness of the respective arrows. *A*, the complete circulation. *B*, the heart alone. Note the numerous small foramina in the interatrial septum as compared with the one larger foramen ovale in the Mammal. The right atrio-ventricular valve is also different from either of the mammalian valves (Fig. 336). With the substitution of the placenta for the allantois, essentially the same type of circulation with the separation of the two classes of blood has been alternately denied and claimed in the case of the Mammal ever since Harvey. For a complete discussion of this controversy see the text. It seems now to be settled as shown.

Because this is an embryonic stage the sixth arches are connected with the aorta. Being a Bird the right sixth unites with the aortic extension of the right fourth arch through the right ductus Botalli. In the Mammal it would be the left. At hatching both ducts of Botallo will close and later atrophy (Coughlin and Walker, '53).

refinements was repeated by Kellogg on both the Pig and the Dog in 1928, and later by others with similar results. Therefore, it was reasonably concluded by both investigators that there had been a thorough mixture of the two classes of blood in the right atrium. And so the question seemed to be finally answered.

Regardless of all this seemingly overwhelming evidence in favor of the theory of mixture, however, many embryologists were still intrigued by the hypothetical desirability of a separation if it could only be proven. Consequently they have once more returned to the attack with both similar and improved techniques, and with most interesting results. In the first place Windle and Becker ('40) using the Cat and Guinea Pig, injected india ink instead of cornstarch. This probably did not reduce the velocity of flow as did cornstarch, thus providing more normal conditions, and their results supported the separation theory of Sabatier. Next, in 1941, Barclay, Barcroft, Barron, Franklin, and Prichard performed the most ingenious experiment yet devised. They injected inert material, opaque to X-rays, into the blood stream of living Sheep fetuses. Then by means of X-ray moving pictures they showed that there is a fairly complete separation of the anterior and posterior streams in the right atrium. This brilliant experiment, especially if confirmed, would seem to be conclusive. Finally, Whitehead ('42) has made a model of an embryo Cat heart in neoprene by the reconstruction method. With it he has demonstrated that the key to the separation of the streams entering its right atrium is the pressure at which each stream enters. He, moreover, believes that the pressures with which the blood streams do enter the actual Cat heart are such as to separate them. Thus the matter rests at the date this book is written, and we are back once more to the purely hypothetical conclusions of 1798.

However this may be in the embryo, it is of course certain that in the adult Bird or Mammal the completely aerated blood from the lungs (arterial blood) is normally entirely separated in the heart and arterial circulation from the venous blood. To achieve this at, or shortly after, the hatching of the Bird or the birth of the Mammal, all that is necessary is the closure of the interatrial openings, or opening, in the septum and the occlusion of the ducts of Botallo (one duct in the Mammal).

Considering the matter of the septum first, it will be recalled that by the end of the sixth day in the Chick this structure was closed except for the existence of numerous foramina. During the embryonic life of the Bird these foramina are kept open according to current theory in the

following manner: The pressure on the septum from the side of the right atrium greatly exceeds that from the left side because of the relatively small amount of blood being returned to the left atrium from the non-functioning lungs. Hence the septum tends to belly out to the left, and to remain in a stretched condition with the foramina wide open. In the Bird, as indicated below, the lungs start functioning to some degree two or three days before hatching takes place. Hence the vessels of these developing organs receive more and more blood, and the pressure on the two sides of the septum is gradually equalized. This causes it to straighten out, the stretch is taken out of it, and as a consequence its wall thickens and the foramina are functionally closed. Later the tissue about the former openings presumably becomes entirely fused. The mechanism in the Mammal is somewhat different, but is supposed also to depend on an equalization of pressure in the two atria, and a functional closure of the single interatrial opening. The details of the process in this class will be discussed further in connection with the Pig.⁴

The closure of the duct of Botallo (arteriosus), at least in the Mammal where it has been most studied, is apparently brought about by the contraction of muscle fibers within its walls. This has been rather cleverly demonstrated in the Guinea Pig by Kennedy and Clark ('41). Under anesthesia living, almost full term, fetuses were removed from the uterus while leaving the umbilical cords attached. The fetuses themselves were then opened so that the heart could be observed. When such a fetus was in the air it would breathe, and the duct of Botallo could be seen to close. When it was immersed in normal saline the embryonic respiratory situation was restored, and the duct of Botallo would promptly reopen. This could be repeated several times. Thus the closure would appear to be a result of the stimulus of breathing. Within a month or so after normal birth, however, the walls of the duct have grown together, and the structure is reduced to a cord.

In conclusion of this topic it may be noted that in man either a defect in the interatrial septum or a persistently patent duct of Botallo are among the causes of infantile cyanosis, "blue babies." Where a patent

⁴ The sudden functioning of the lungs as a factor in increasing the blood flow from them to the heart in the case of the Mammal has been questioned for the Cat and Guinea Pig by Abel and Windle ('39). These authors claim that there is already a good deal of circulation here at term, and that subsequent increase is gradual. A similar situation is also claimed for other Mammals, including Man (Patten, '46). As noted the condition in the Bird is such that in that case gradual initiation of lung function, and hence of change in the course of the blood, must always occur.

duct is the primary defect, it may be remedied by tying off this vessel. A failure in septal closure, however, is more difficult to cope with. Yet now even this may be greatly helped by a clever operation which involves rerouting part of the aortic blood to the lungs.

The Subclavian Arteries. — The primary subclavian arteries arise as outgrowths from the eighteenth segmental arteries. On the fifth day, however, an anteriorly growing branch of each primary artery connects with the respective third aortic arch, which as indicated eventually becomes a part of the common carotid (Bakst and Chafee, '28; Figs. 233 and 235). These new branches then develop, while the original connections with the dorsal aorta through the segmental arteries become atrophied. Thus the *permanent subclavians* eventually arise from the carotids in the Bird. These arteries, of course, supply the wings, and in so doing, develop various branches. It will not be advisable, however, to follow them further in detail.

The Remaining Arteries. — The only other major arteries whose development has not already been indicated in the account of the fourth day, are the coeliac, the anterior mesenteric and the posterior mesenteric. The *coeliac* arises from the anterior part of the dorsal aorta, and supplies the stomach, gizzard and part of the intestine. The *anterior mesenteric* originates as an outgrowth from the single vitelline artery close to the place where the latter leaves the aorta, and supplies the intestine. Lastly the *posterior mesenteric* develops from the aorta slightly caudal to the kidneys, and supplies the rectum and cloaca. These three arteries appear during the fifth and subsequent days (Fig. 237).

The Veins.

The Vitelline Veins. — At the end of the fourth day, a second venous ring had been formed about the intestine by a fusion of the vitelline veins for a short distance beneath it. This second ring was beginning to be destroyed by the disappearance of its right side, and during the fifth day, this side is completely obliterated. From a review of the previous development of this region, it will be evident that the condition of the vitelline veins at this point has now become as follows. The two veins unite just in front of the anterior intestinal portal, and ventral to the intestine, to form a single trunk, which is really a posterior continuation of the ductus venosus. This trunk runs forward beneath the intestine for a short distance, and then curves upward and to the left. It next turns sharply to the right and crosses over the intestine dorsally; finally it bends immediately downward and again runs anteriorly to pass into the

liver (Fig 211, *E*). During subsequent stages as the anterior intestinal portal continues to move backward, it is closely followed by the fusion of the vitelline vessels. Indeed before very long this fusion passes beyond the region of the intestinal portal, and thus the single ductus venosus, or vitelline trunk, comes to extend a considerable distance into the umbilicus before dividing into its two branches.

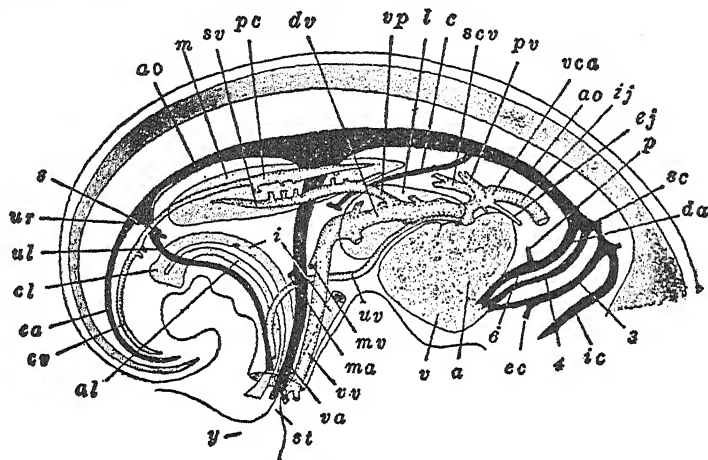


Fig. 237. — Diagrammatic lateral view of the chief embryonic blood vessels of the Chick, during the sixth day. From Kellicott (*Chordate Development*). After Lillie.

a. Atrium. al. Allantoic stalk. ao. Dorsal aorta. c. Coeliac artery. ca. Caudal artery. cl. Cloaca. cv. Caudal vein. da. Ductus arteriosus. dv. Ductus venosus. ec. External carotid artery. ej. External jugular vein. i. Intestine. ic. Internal carotid artery. ij. Internal jugular vein. l. Liver. m. Mesonephros. ma. Mesenteric artery. mv. Mesenteric vein. p. Pulmonary artery. pc. Posterior cardinal vein. pv. Pulmonary vein. s. Sciatic artery. sc. Subclavian artery. scv. Subclavian vein. st. Yolk-stalk. sv. Subcardinal vein. ul. Left umbilical artery. ur. Right umbilical artery. uv. Left umbilical vein. v. Ventricle. va. Vitelline artery. vca. Anterior vena cava (anterior cardinal vein). vp. Posterior vena cava. vv. Vitelline vein. y. Yolk-sac. 3, 4, 6. Third, fourth, and sixth aortic arches.

The Hepatic Portal System. — It will be recalled that within the liver the ductus venosus receives numerous capillaries. These capillaries increase during the fifth day, while at the same time the main channel of the vein within the liver begins to disappear. This is brought about through the gradual occlusion of this channel by means of strands of the hepatic substance which grow into and across it. On the fifth day also, a vessel starts to develop in the dorsal mesentery of the gut; it is the *mesenteric vein*, and presently acquires a connection with the vitelline trunk at about the region of the pancreas. By the seventh day the

occlusion of the main part of the ductus venosus within the hepatic substance has been completed. From now on, therefore, the blood enters the liver by the remaining posterior half of this vein, is distributed through the hepatic capillaries, and is finally collected again to enter the now separate anterior half of the same vessel through two main branches. When development has reached this stage the posterior half of the ductus venosus may be termed the *hepatic portal vein*, which receives the mesenteric vessel as its chief tributary. The two branches entering the anterior half of the ductus venosus, upon the other hand, constitute the *hepatic veins* (Fig. 211, *F*).

Upon the fifth and immediately subsequent days the blood which enters the liver circulation is largely from the yolk-sac. Before long, however, the mesenteric vein has begun to send out branches which develop simultaneously with the various digestive organs and spleen. Thus these organs send an ever-increasing supply of blood through the hepatic portal vein to the liver. When the yolk-sac finally disappears they become the sole source of the blood which passes through the hepatic capillaries. The complete system of circulation which is developed in this manner is then called the *hepatic portal system*.

The Fate of the Cardinals and Development of the Caval and Renal Veins. — On the fourth day, the subcardinals lying ventral to the mesonephros have direct connections with the posterior cardinals lying dorso-lateral to it. Upon the fifth day, however, these connections are severed and new ones established through capillaries within the mesonephric substance. At the same time, the subcardinals fuse with one another near their anterior ends, and the connection of the right one with the posterior end of the vena cava inferior (established on the fourth day) becomes larger (Fig. 238). Thus a part of the blood in the posterior cardinals now passes through the mesonephros and by way of the subcardinals and vena cava inferior to the heart. In other words, there is in the embryo of the Bird a typical *renal portal* circulation. On the fifth day also, or late upon the fourth, the *subclavian veins* begin to develop in connection with the fore-limb buds. They arise as branches of the posterior cardinal veins, a short distance behind the junction of the latter with the Cuvierian ducts.

Upon the sixth day, the section of each posterior cardinal between the entrance of the respective subclavian vein and the anterior end of the mesonephros disappears, thus forcing all the blood from the posterior part of the body to traverse the renal portal channels. In this manner also that portion of each posterior cardinal anterior to the entrance of

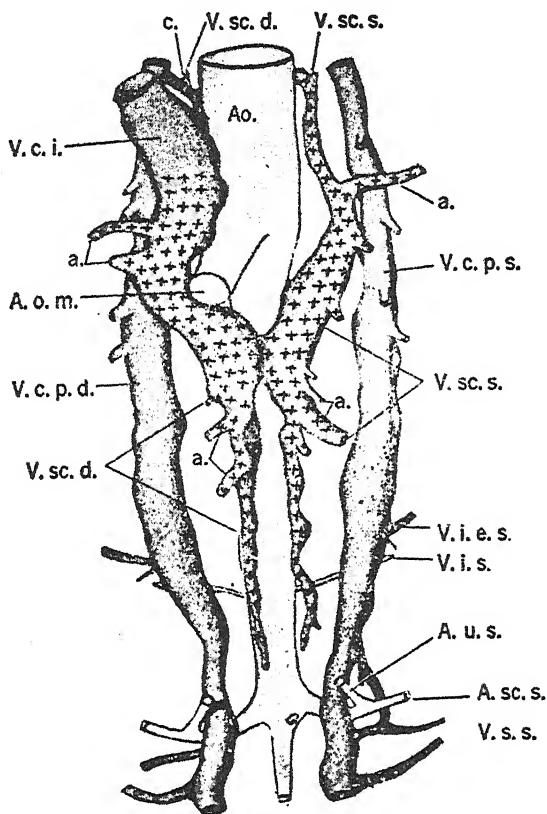


Fig. 238.—Reconstruction of the venous system of a Chick of 5 days. Ventral view. From Lillie (*Development of the Chick*). After Miller.

a. Mesonephric veins. Ao. Aorta. A.o.m. Omphalomesenteric artery. A.u.s. Left umbilical artery. A.sc.s. Left sciatic artery. V.c.p.d.s. Right and left posterior cardinal veins. v.c.i. Vena cava inferior. V.sc.d.,s. Right and left subcardinal veins.

the subclavian becomes simply the proximal part of the latter vessel. From this time on, the ducts of Cuvier, which now receive the jugulars (anterior cardinals) and subclavians, may be termed the *anterior or superior caval veins*. At about this stage also, the anterior portion of the ductus venosus, which receives the two hepatic veins and the posterior vena cava (vena cava inferior), may be said to have become merely the anterior end of the latter vessel. Thus the posterior caval vein, like the

two anterior cavals, now opens directly into the right atrium (Fig. 237).

While the above changes are occurring subsequent to the fifth day, there are a pair of new veins arising in connection with the metanephros

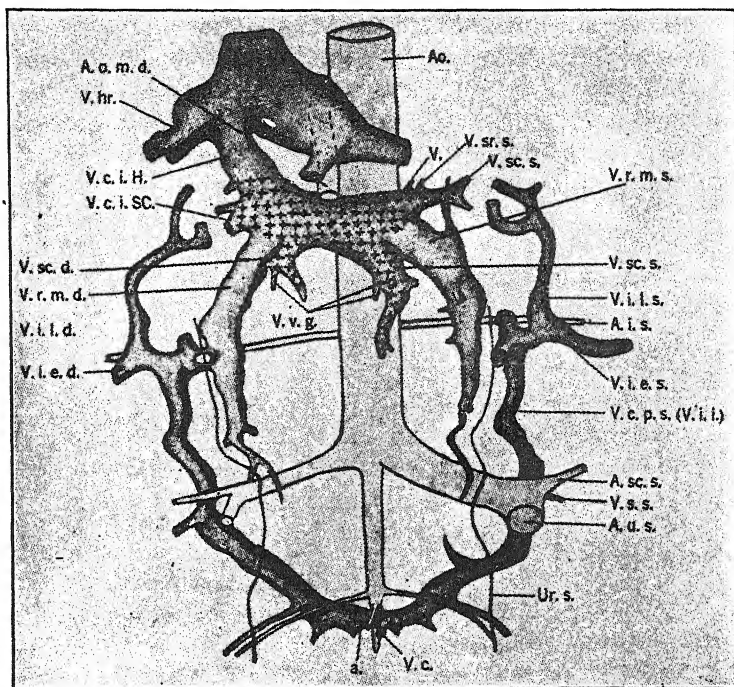


Fig. 239. — Reconstruction of the venous system of a sparrow embryo, corresponding to a chick of about 14 days. From Lillie (*Development of the Chick*). After Miller.

V.c.i.H. Intra-hepatic part of the vena cava inferior. *V.c.i.SC.* Part of the vena cava inferior derived from the subcardinal vein. *V.v.g.* Genital veins. *V.i.e.d.,s.* Right and left vena iliaca externa. *V.i.i.* Vena iliaca interna, (or *V.c.p.s.* Posterior part of the left cardinal). *V.i.l.d.,s.* Right and left vena intervertebralis lumbalis. *V.r.m.d.,s.* Right and left great renal veins.

or permanent kidney. These are the *renal veins* which presently take blood from the permanent kidney to the anterior fused portion of the subcardinals (now really the posterior part of the posterior vena cava). Just anterior to the kidney these renal veins also later establish direct connections with the posterior cardinals. Thus a new channel is formed for the blood from the posterior part of the body via the cardinals and the anterior portion of the new renal veins to the posterior vena cava (Fig. 239). At the same time that this is occurring, the mesonephros to-

gether with the renal portal system is disappearing. While the latter exists, however, it is essentially similar to the permanent system of the same name in the Frog and other more primitive Vertebrates, thus affording an excellent example of recapitulation. It remains to note that the hinder portions of the posterior cardinal veins persist in the adult Bird as the *iliac veins*, receiving branches from the hind-limbs. Also in subsequent stages, branches from the cardinals fuse with one another medially at the posterior end of the body and give rise to the *caudal vein*.

THE BODY CAVITIES

From previous discussion, it will be recalled that the space surrounding the heart has been designated as the pericardial cavity. Up to this time, however, there has been no mention made of any separation of this cavity from the *peritoneal* or general body cavity behind it. It now remains to describe how this separation is effected, together with the simultaneous closing off of a third space, the pleural cavity (see below). It will then be possible in conclusion to show also how the walls of the pericardial cavity come to form the independent pericardial sac of the adult bird.

THE SEPARATION OF THE PERICARDIAL, PERITONEAL AND PLEURAL CAVITIES

The separation of the peritoneal and pericardial cavities is chiefly brought about by the development of a partition known as the *septum transversum*. This so-called septum in turn is composed of three parts, two of which have already been mentioned. The entire septum then is made up as follows: First, there is a median mass consisting of the liver and the sinus and ductus venosus, together with the dorsal and ventral ligaments which unite the liver to the gut and for a time to the ventral body wall. Second, there are the lateral mesocardia extending obliquely in an anterior and lateral direction from the median mass to the body walls. Above and below the lateral mesocardia, the pericardial cavity still communicates posteriorly with the peritoneal or general body cavity. About the fifth day, however, the ventral communication begins to be closed. This is accomplished by the development of the third part of the septum transversum, i.e., the *lateral closing fold*, extending from the mesocardia to the ventro-lateral body wall. By the eighth day, this closure is complete. In the meantime, the lungs have been developing in

the portion of the peritoneal space which extends forward above the pericardial cavity. This space may be termed *pleural cavity*, and at this time (fifth day) the oblique lateral mesocardia have not yet entirely separated it anteriorly from the pericardial cavity beneath it; posteriorly also it still communicates with the general body cavity. Presently, however, with the further development of the lateral mesocardia and other parts, the opening between the pleural and pericardial cavities is closed, and a closure of that between the pleural and body cavities soon follows (tenth day). This latter is effected by the *pleuro-peritoneal septum*, which arises as an outgrowth from the sides of the esophagus. The median pericardial cavity is thus bounded dorsally largely by the mesocardia, laterally and ventrally by the peritoneum of the body wall, and posteriorly chiefly by the median mass of the septum transversum.

THE ESTABLISHMENT OF THE DEFINITIVE PERICARDIUM

Eventually, however, the tissue upon the front of the median mass becomes thickened and splits into two sheets. The anterior sheet then becomes the posterior wall of the pericardium, the posterior sheet covers the face of the liver, and the general body cavity extends between them. At the same time, the latter cavity is also pushing forward beneath and at the sides of the present pericardium, and as it does so, it apparently splits the peritoneum of the body wall into two layers. The outer layer forms the peritoneum of the general body cavity in this region, and the inner layer constitutes the ventral and lateral wall of the pericardium proper. In this manner, the final pericardial wall or *definitive pericardium* of the adult bird comes to surround the heart as a relatively independent sac with a portion of the liver extending beneath it.

THE URINOGENITAL SYSTEM

THE EXCRETORY SYSTEM

The Mesonephros. — During the fifth day, the increase in the number of the mesonephric tubules ceases, while the organ becomes more active as a kidney. For a couple of days subsequent to this, however, the tubules continue to grow in length, thus greatly increasing the bulk of the organ. Degeneration begins about the eleventh day, and from then on, the metanephros aids in performing the excretory functions which it later entirely takes over.

The Metanephros. —

At the end of the fourth day, the diverticulum (ureter) from the posterior end of the Wolffian duct had just appeared, and the nephrogenous tissue immediately behind the mesonephros had degenerated. During the fifth day, the above diverticulum, accompanied by the nephrogenous tissue posterior to the region of degeneration, grows forward somewhat, and begins to branch dichotomously (Fig. 240, representing a slightly later stage). Its position in this region is adjacent to the posterior cardinal vein, upon the median side of the latter and above the Wolffian duct. The accompanying nephrogenous tissue lies, in turn, adjacent to the median side of the diverticulum, so that the latter, i.e., the diverticulum, lies between the vein and the tissue. The nephrogenous tissue, which is in immediate contact with the diverticulum and its branches, is called the *inner zone*. Lastly this inner zone is covered on its median side by a layer of dense mesenchyme which

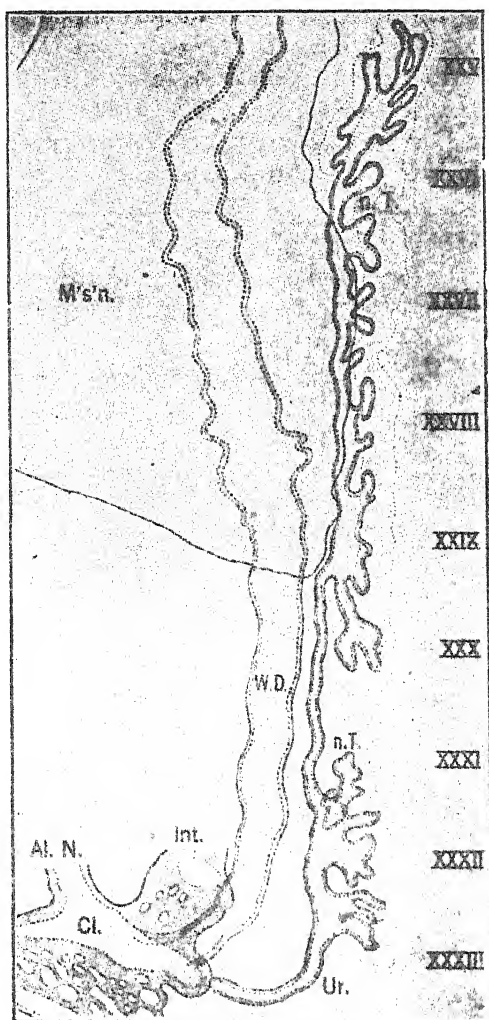


Fig. 240. — Profile reconstruction of the Wolffian duct and primordium of the metanephros of a Chick embryo of 6 days and 8 hours. From Lillie (*Development of the Chick*). After Schreiner.

XXV to XXXIII, the twenty-fifth to thirty-third somites. *AL.N.* The neck of the allantois. *Cl.* The cloaca. *Int.* The intestine. *M's'n.* The mesonephros. *n.T.* The nephrogenous tissue of the metanephros included within the dotted lines. *W.D.* The Wolffian duct. *Ur.* The ureter.

differentiates in advance of the growing nephrogenous element and diverticulum. It is called the *outer zone* (Fig. 241).

During subsequent days, the posterior end of the mesonephric duct bearing the metanephric diverticulum (ureter) is drawn into the cloaca, and thus the ureter acquires an opening separate from that of the mesonephros (Fig. 240). The other end of the metanephric duct, with its

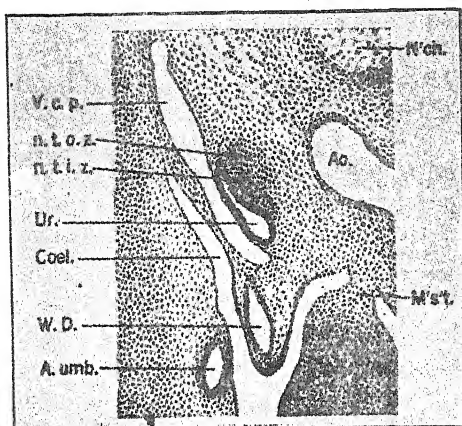


Fig. 241. — Transverse section through the ureter and metanephrogenous tissue of a five-day Chick. From Lillie (*Development of the Chick*).

A.umb. Umbilical artery. *Coel.* Coelom. *M's't.* Mesentery. *n.t.i.z.* Inner zone of the nephrogenous tissue. *n.t.o.z.* Outer zone of the nephrogenous tissue. *Ur.* Ureter. *V.c.p.* Posterior cardinal vein. *W.D.* Wolffian duct.

inner and outer zones, meanwhile, grows still further forward till it reaches the region of the mesonephros, and then continues on dorsal to that organ, nearly to its anterior extremity. The inner zone of this tissue everywhere gives rise to the secreting tubules and glomeruli of the permanent kidney in a manner very similar to that described for the mesonephros. These tubules then connect with the dichotomous branches of the metanephric duct, which thus function as collecting tubules, while the duct it-

self becomes the ureter of the adult. Eventually the outer zone helps to form a connective tissue covering for the entire organ.

THE REPRODUCTIVE SYSTEM

The Gonads in the Male. — During the fourth day, it is impossible to distinguish sex. Occasionally on the fifth day, but more generally and definitely on the sixth, the distinction becomes possible by the fact that in the female the left gonad is slightly larger than the right. This is apparently due to the fact that the right gonad usually possesses relatively little cortex, and fewer germ cells. These latter facts according to Witschi ('35) are correlated. The left gonad in the female possesses more cortex because of the female chromosomal complex and the excess cortex this worker thinks acts as an inductor to attract more germ

FIFTH DAY: THE REPRODUCTIVE SYSTEM 469

cells. Be this as it may, in the male, which is to be considered first, there is virtually no difference between the gonads, and therefore the description of one will suffice for both.

It has been indicated in the introductory discussion of germ cells in general that the primordial germ cells of the Chick are said to be first

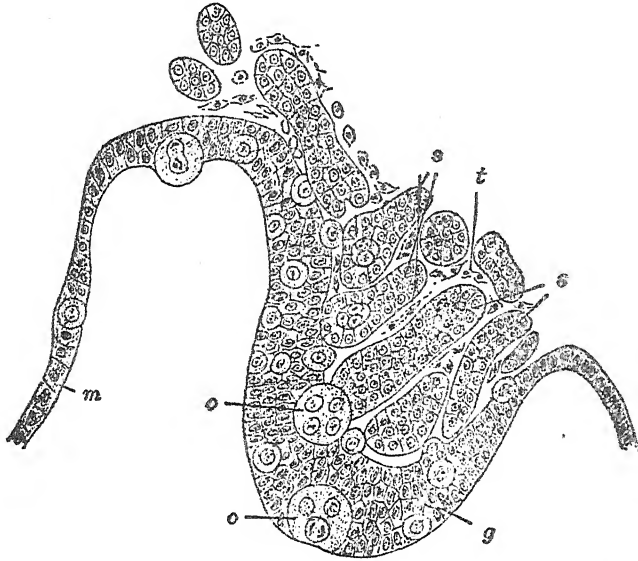


Fig. 242. — Section through the gonad of a Chick, the middle of the fifth day, showing the sexual cords growing inward from the germinal epithelium. The connections of many of the cords with the epithelium have been cut across. From Kellicott (*Chordate Development*). After Semon.

g. Germinal epithelium. m. Epithelium of the mesentery (peritoneum). o. Primordial germ cells. s. Sexual cords. t. Connective-tissue stroma.

discernible well outside the embryo. Indeed, according to Swift ('14) and Goldsmith ('28, '35), these cells are first found at the primitive streak stage in the zone of junction lateral to the proamnion. From here they are carried by the blood stream to the vicinity of the germinal epithelium, whence by amoeboid movements they enter this epithelium during the fourth and fifth days.

More recently, so far as the representatives of these cells which actually reach the germinal epithelium are concerned, their initial transfer by means of the blood stream has been denied (Stanley and Witschi, '40). These authors admit that primordial germ cells are indeed found

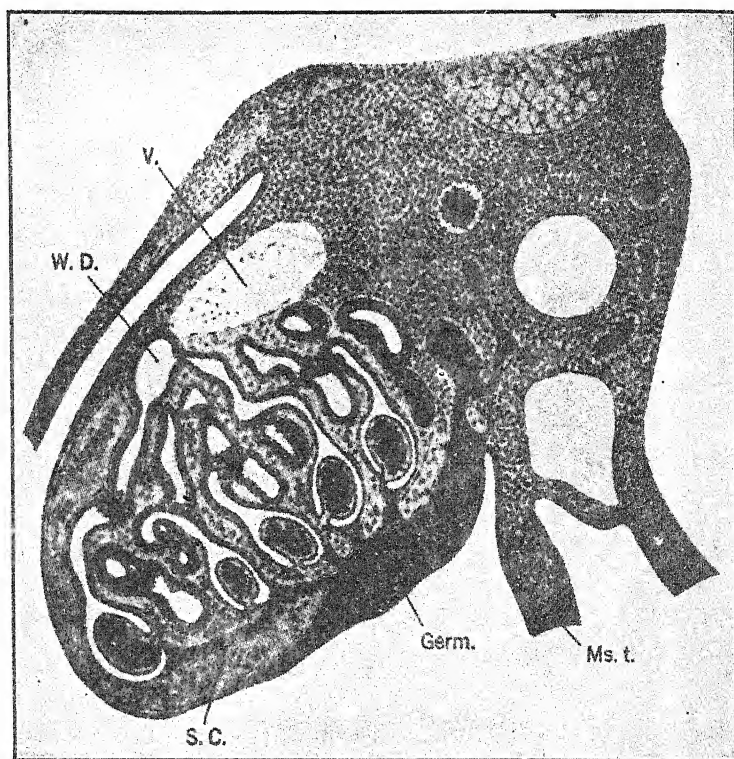


Fig. 243. — Cross-section through the genital primordium of *Limosa aegocephala*. From Lillie (*Development of the Chick*). After Hoffmann, from Felix and Buhler. The stage is about similar to that of a Chick embryo of 4½ days, and shows the rete cords extending from the Malpighian tubules to the germinal epithelium. The latter appears in the figure as a dark mass on the right ventral side of the mesonephros next to the mesentery. Three primordial germ cells (light colored) are visible in it.

Germ. Germinal epithelium. Ms.t. Mesentery. S.C. Rete cord. V. Posterior cardinal vein. W.D. Wolffian duct.

in the blood in early stages, but claim that they are only cast offs, never destined to enter the gonads. According to them all movement of such cells really on their way to the germinal epithelium is by passive shifting accompanying growth and rearrangement of parts, and later by active migration as indicated.⁵ Be this as it may, by the fifth day the germinal epithelium with the primordial germ cells in it is being drawn

⁵ It must be further noted that according to Firket ('20) and others all, or most, of these so-called primordial germ cells in the Chick, as in the Albino Rat, ultimately degenerate and are replaced by definitive germ cells derived from the germinal epithelium itself.

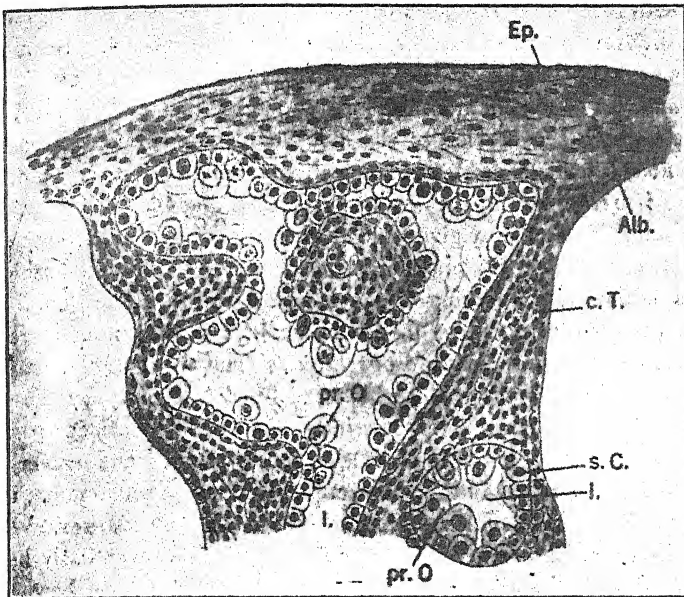


Fig. 244. — Cross-section through the periphery of the testis of a just hatched Chick. From Lillie (*Development of the Chick*). After Semon. The sexual cords have acquired a lumen, and the walls of the canals thus formed are lined within by the spermatogonia. Next to the latter come a layer of supporting or Sertoli cells. The connective tissue (stroma) lying between the sexual cords (now seminiferous tubules) connects at the periphery of the testis with the special layer of connective tissue (albuginea) which covers the entire organ beneath the thin outermost layer of coelomic epithelium.

Alb. Albuginea. *c.T.* Connective tissue of the stroma, or septulae testis. *Ep.* Remains of the germinal epithelium now forming the outermost or serous covering of the testis. *l.* Lumen of the sexual cords. *pr.O.* Spermatogonia. *s.C.* Sexual cord, lined by supporting cells and spermatogonia.

somewhat on to the ventro-median surface of the mesonephros. Meanwhile from the capsules of the Malpighian bodies of that organ, strands of cells begin to grow out through the loose mesenchyme to the germinal epithelium. These strands are the *rete cords*, and are destined to form the *vasa efferentia* which help to connect the future tubules of the testis with the vas deferens (see below). At about this period also the germinal epithelium begins to send processes inward among the mesenchyme cells and the rete cords. These new strands of tissue of epithelial origin are the *sexual cords*, which contain primordial germ cells (Figs. 242, 243). Up to this point the condition of the male gonad is virtually iden-

tical with that of the female. From now on, however, the former begins to be differentiated to form the adult *testis* in the following manner:

The sexual cords become separated from the epithelium, and increase in number so as to constitute the bulk of the organ (seventh day), while the rete cords are pressed to the side nearest the mesonephros. Presently also (eleventh day) the mesenchyme, which has been scanty, begins to increase among the sexual cords, forming the connective tissue or *stroma*. Eventually it gives rise further to a layer, the *albuginea*, lying between these cords and the reduced sheet of epithelium which remains as the outer covering of the gonad. Meanwhile the sexual cords themselves (twentieth day) begin to acquire a lumen, and are thus transformed into the *seminiferous tubules*. The walls of the latter are composed of supporting cells which are lined internally by the multiplying primordial germ cells. The latter may now be termed spermatogonia, from which arise in turn the spermatocytes and sperm (Fig. 244). It is to be noted in this connection that the spermatogonia, unlike the oögonia in the Bird, continue to divide throughout the sexual life of the individual. The ends of the seminiferous tubules eventually become connected with the rete cords which, as indicated above, become the vasa efferentia. These in turn connect with the modified mesonephric tubules in the anterior or sexual half of that organ, which thus becomes the *epididymis*. The posterior and non-sexual portion of the mesonephros which remains becomes a vestige known as the *paradidymis*.

The Gonads in the Female. — Although differences in sex may be indicated by the disparity in the size of the gonads as early as the fifth day, there is little else to distinguish male from female at this time. The description of the testes up to this point will, therefore, suffice also for the ovaries. The right and left *ovary*, however, are different in the Bird, and this difference appears at an early stage.

In the left ovary, following the sixth day, a secondary set of sexual cords, the *ovigerous cords*, grow inward from the germinal epithelium, and again carry primordial germ cells. The new cords press the original or primary cords into the medullary region, and the germinal cells in the latter cords degenerate. In the right ovary no such secondary growth occurs, and under normal conditions the primary cords develop only slightly, the whole structure remaining rudimentary unless artificially stimulated by injected male hormone to form a testis. In the left ovary, however, the secondary or ovigerous cords soon break up into nests, each containing at least one germ, surrounded by remaining epithelial cells which form its *follicle*. From this point on, the young egg cell begins to grow,

and it may, therefore, be termed an oöcyte (Fig. 245). This growth period is reached earlier by some ova than by others, but the oögonial or multiplication stage ceases for all about the time of hatching. The anterior portion of the mesonephros, which in the male forms the epididy-

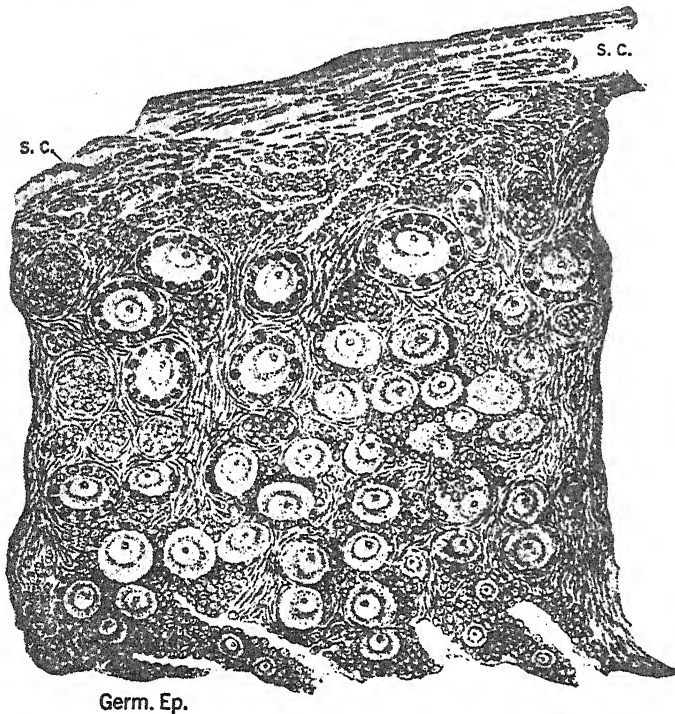


Fig. 245. — Cross-section of the ovary of a fledgling of *Numenius arcuatus* 3-4 days old. The germinal epithelium is below. From Lillie (*Development of the Chick*). After Hoffmann. Note numerous oöcytes surrounded by a single layer of follicle cells.

s.c. Sexual cords degenerating. Germ. Ep. Germinal epithelium producing ovigerous cords.

mis, remains as a minute rudiment, the *epoöphoron*. The paradidymis of the male is sometimes evident in the hen as a still smaller vestige, the *paroöphoron*.

The Gonoducts in the Male. — It has already been stated that in the male, the Wolffian ducts become the *vasa deferentia* or sperm ducts of the adult. They connect with the testes through the vasa efferentia and epididymis. Late in development, they become muscular and somewhat convoluted, with a dilation at their posterior extremities.

The Gonoducts in the Female. — As has been stated, the oviducts begin development on the fourth day as the tubal ridges, one on the lateral side of each mesonephros adjacent to the respective Wolfian duct. During the fifth day, a groove-like invagination develops along the an-

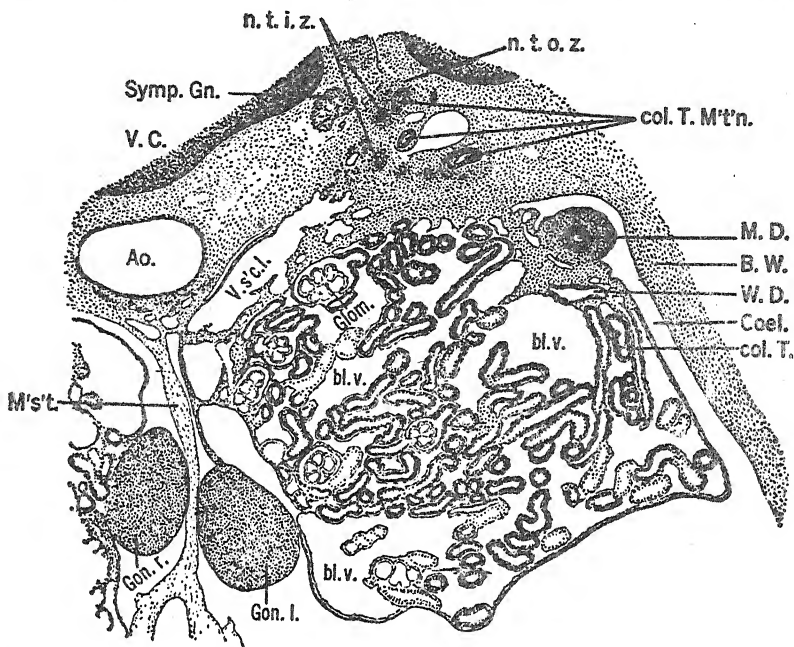


Fig. 246. — Transverse section through the metanephros, mesonephros, gonads and neighboring parts of an 8-day Chick. From Lillie (*Development of the Chick*).

Ao. Aorta. bl.v. Blood vessels. B.W. Body-wall. Coel. Coelom. Col.T. Collecting tubule of the mesonephros. col.T.M'n. Collecting tubules of the metanephros. Glom. Glomerulus. Gon.l. Left gonad. Gon.r. Right gonad. M.D. Müllerian duct. M's.t. Mesentery. n.t.i.z. Inner zone of nephrogenous tissue (metanephric). n.t.o.z. Outer zone of the nephrogenous tissue. Symp.Gn. Sympathetic ganglion of the twenty-first spinal ganglion. V.C. Centrum of vertebra. V.s.c.l. Left subcardinal vein. W.D. Wolfian duct.

terior portion of each ridge, and the lips of the groove fuse with one another to make a tube open at its anterior end. This tube which is quite short, then grows backward independently between the remaining tissue of the ridge and the Wolfian duct (Fig. 246).

Subsequent development is as follows: By the eighth day each duct has reached the cloaca, but does not open into it. At this time, there begins the atrophy of both ducts in the male and of the right duct in the

female, accompanied in both sexes by the disappearance of the remains of the tubal ridges. The left duct in the female, however, gradually enlarges and differentiates the infundibulum and glandular portions characteristic of the adult. It does not, however, effect an entrance into the cloaca until the hen is about six months old (Lillie after Gasser). It always remains attached to the body wall and the rudiments of the mesonephros by a ligament or mesentery-like fold.

THE ADRENALS

During the fifth day, the cortical substance, noted as arising on the fourth day, increases in amount, and comes into relation with the Malpighian capsules. On the sixth day it begins to be arranged in definite cords, which during subsequent days increase in size and number, while at the same time innervation of the organ begins. On the eighth day this mass of cords is becoming penetrated by blood sinuses and by the medullary material previously indicated. Within the latter, "chromaffine" cells are being differentiated, and eventually this medullary material also acquires a cord-like arrangement.

HATCHING

It will be recalled that originally the embryo was orientated with its long axis transverse to that of the shell, and with the head away from the observer when the large end of the shell is to the observer's left. Between the fifth and ninth days the position of the embryo varies considerably, and changes from time to time due to active contractions of the amnion. By the tenth day, however, a normal embryo again assumes the original position relative to the shell. But at this stage it is nearer to the large end of the latter, and lies with its back against the yolk-sac instead of either its ventral parts or its side. In this position of course its legs are pressed against the shell. Next, aided by contractions of the amnion, the yolk-sac is moved first toward the small end of the shell, and then up over the ventral side of the embryo. This movement is usually completed by the thirteenth or fourteenth day. During the next three or four days the yolk-sac moves on over the ventral side of the embryo until the now partially emptied and flabby sac occupies the large end of the shell. As this is occurring the embryo by means of vigorous wriggling turns itself so that when the process is completed its tail is at the small end of the shell, i.e., the long axis of the embryo and shell have

now become parallel. According to the schedule indicated this condition is finally achieved on the seventeenth or eighteenth day.⁶ The next step involves the piercing of the egg membrane by the beak so that breathing of air from the air chamber can begin. Some respiratory movements may occur, however, even before this, there being by this time small amounts of air in other parts of the egg. As respiration starts the amnion and allantois dry up and become detached, while movements of the abdomen draw the remains of the yolk-sac within the body. At the same time the necessary circulatory changes are occurring within the embryo as already described. About the last hour before hatching on the twenty-first day the Chick starts a vigorous counter clock-wise rotation within the shell aided by strong thrusting movements of the legs. Presently as a result of the thrusting of the legs and the stretching of the neck the shell is broken into two parts and the Chick is hatched.

The foregoing description of later positional changes and hatching is taken from the detailed account by Kuo ('32). One interesting feature which is not mentioned by this author, however, is the so-called egg tooth. This is a sharp cone shaped point of horny material developed on the dorsal side of the beak, and is said by other writers to function in chipping the shell. At all events it is a transitory structure lost soon after hatching.

SUMMARY OF THE CONDITION AT THE END OF THE FIFTH DAY OF INCUBATION

I. THE EXTERNAL APPEARANCE

The *cervical flexure* has reached its maximum development, the *third visceral cleft* has closed, and the future *neck* is slightly indicated. The *limb buds* are beginning to appear jointed. The nasal apertures are separated into internal and external *nares* and the *beak* and *mandible* are just starting to form.

II. THE FEATHERS

A depression develops in the skin. At its bottom a slight outgrowth arises consisting of a core of mesoderm, the *pulp*, with a covering of the Malpighian layer and a thin outer layer of cornified epithelium. This outgrowth is the *papilla*. The papilla emerges above the depression, and is known as the *feather germ*. With further growth and the throwing off

⁶ Waters ('35) says usually not until the nineteenth or twentieth day.

of the cornified cells the Malpighian layer becomes folded and modified to form the quill and barbs of a *feather*. Feather germs appear in the Chick on about the eighth day.

III. THE SKELETON

The definitive or *vertebral* segmentation of the *mesenchymal sheath* about the notochord and nerve cord has become more marked, while all the sclerotomal tissue is becoming membranous. These membranous condensations are especially evident in certain regions, representing parts of the *future vertebrae neural arches* and *costal processes*. Mesenchymal concentrations representing the *limb bones* and the parts of the *pectoral* and *pelvic girdles* are also visible. The various parts of the *primordial cartilaginous cranium* and *visceral skeleton* are discernible at this time as concentrations of mesenchyme about the head

IV. THE ALIMENTARY TRACT

The Fore-gut Region. — The *third visceral cleft* closes, the *lung rudiments* have grown posteriorly somewhat through a mass of developing mesoderm, and faint indications of the *abdominal* and *cervical air sacs* may be present. The *glottis* is partly closed.

The *esophagus* has continued to elongate, the *stomach* is slightly dilated, and a pouch representing the rudiment of the *gizzard* has appeared in connection with it. The *duodenal loop* is barely defined. The *liver* has continued to branch, and some of the branches have acquired lumens. The three *pancreatic diverticula* have also branched somewhat.

The Mid-Gut Region. — The end of the duodenum is marked by a ventral bend, the *duodeno-jejunal flexure*. From here the mid-gut or *small intestine* descends to connect with the yolk-sac, and passes dorsally again to its posterior end, marked by rudiments of the *intestinal caecae*.

The Hind-gut Region. — The *hind-gut* or *rectum* is not materially altered, but the laterally compressed walls of the posterior part of the cloaca have become fused.

V. THE CIRCULATORY SYSTEM

The Heart. — The *alterations* in the *relative positions* of the parts are nearly completed, as are also the *septa* within the heart. The *septum* of the *truncus arteriosus* has formed and that of the *bulbus* has started to develop.

The Arteries. — The portions of the *dorsal aortae* between the third and fourth arches have begun to disappear, and the *left fourth arch* has

also diminished in size. The *subclavian* arteries have become connected with the carotids and the *anterior mesenteric* and *coeliac* arteries are developed.

The Veins. — The right side of the *second venous ring* about the intestine has disappeared, so that in this region there is only a single vitelline trunk. Within the liver, the capillaries of the *ductus venosus* are continuing to develop, while the main channel is atrophying. The *mesenteric vein* has started to form.

The *subcardinals* have lost their original direct connections with the *posterior cardinals*, and have developed new ones through capillaries within each mesonephros. At the same time the subcardinals have fused with one another anteriorly, and by means of the previous connection with the *vena cava inferior*, have thus established a *renal portal system*. The *subclavian* veins have started to develop from the posterior cardinals.

VI. THE BODY CAVITIES

The ventral communication between the *pericardial* and *peritoneal* cavities has begun to be closed by the development of the *lateral closing folds* beneath the *lateral mesocardia*.

VII. THE NERVOUS SYSTEM

In connection with the description of this system in the preceding chapter, it was noted that there are few important developments occurring in it on the fifth day. The following events, however, may be mentioned as having taken place during this period. The *fourth cranial nerves* have originated, and in connection with the ear the rudiments of the *semicircular canals* have appeared. In the eye the mesenchymal part of the *pecten* is increasing, while the lips of the choroid fissure are beginning to overgrow it.

VIII. THE URINOGENITAL SYSTEM

The Excretory System. — The *mesonephric tubules* have ceased to increase in number, but are continuing to grow in length as the organ becomes more active. The *metanephric diverticulum*, accompanied by its *nephrogenous tissues* or *inner zone*, has grown forward and begun to branch, while about the latter the *outer zone* is developing from mesenchyme.

The Genital System. — The *primordial germ cells* have begun to pass into the *germinal epithelium* and the *rete* and *sexual cords* have

started to develop. The male and female gonads are similar except for occasional differences in size between the right and left organs in the female. In both sexes, the *oviducts* are present as small tubes growing toward the cloaca.

IX. THE ADRENALS

The *cortical substance* of the adrenals increases in amount, and comes into relation with the Malpighian capsules.

REFERENCES TO LITERATURE

CHAPTERS VIII, IX, X, XI, XII, AND XIII

- Abel, S. and Windle, F. W., "Relation of the Volume of Pulmonary Circulation to Respiration at Birth," *Anat. Rec.*, LXXV, 1939.
- Abel, W., "Further Observations on the Development of the Sympathetic Nervous System in the Chick," *Jour. Anat. Physiol.*, XLVII, 1912.
- Alexander, L. E., "An Experimental Study of the Role of Optic Cup and Overlying Ectoderm in Lens Formation in the Chick Embryo," *Jour. Exp. Zool.*, LXXV, 1937.
- Asmundson, V. S. and Burmester, B. N., "The Secretory Activity of the Parts of the Hen's Oviduct," *Jour. Exp. Zool.*, LXXII, 1936.
- Bakst, H. and Chafee, F. H., "The Origin of the Definitive Subclavian Artery in the Chick Embryo," *Anat. Rec.*, XXXVIII, 1928.
- Barclay, A. E., Barcraft, J., Barron, D. H., Franklin, K. J., and Prichard, M. M. L., "Studies of the Foetal Circulation and of Certain Changes that Take Place after Birth," *Am. Jour. Anat.*, LXIX, 1941.
- , Franklin, K. J., and Prichard, M. M. L., "*The Foetal Circulation and Cardiovascular System and the Changes That They Undergo at Birth*," Oxford, 1944.
- Barron, D. H., "Observations on the Early Differentiation of the Motor Neuroblasts in the Spinal Cord of the Chick," *Jour. Comp. Neur.*, LXXXV, 1946.
- Barry, A., "The Intrinsic Pulsation Rates of Fragments of the Embryonic Chick Heart," *Jour. Exp. Zool.*, XCI, 1942.
- Bartelmez, G. W., "The Bilaterality of the Pigeon's Egg: A study in Egg Organization from the First Growth Period of the Oöcyte to the Beginning of Cleavage. Part I," *Jour. Morph.*, XXIII, 1912. — "The Relation of the Embryo to the Principal Axis of Symmetry in the Bird's Egg," *Biol. Bull.*, XXXV, 1918.
- Beard, J., "The Development of the Peripheral Nervous System of Vertebrates: Part I. Elasmobranchii and Aves," *Q. J. M. S.*, XXIX, 1888.
- Blount, M., "The Early Development of the Pigeon's Egg, with Especial Reference to the Supernumerary Sperm Nuclei, the Periblast, and the Germ Wall," *Biol. Bull.*, XIII, 1907.
- Boyden, E. A., "An Experimental Study of the Development of the Avian Cloaca, with Special Reference to a Mechanical Factor in the Growth of the Allantois," *Jour. Exp. Zool.*, XI, 1924.
- Bueker, E. D., "The Influence of a Growing Limb on the Differentiation of Somatic Motor Neurons in Transplanted Avian Spinal Cord Segments," *Jour. Comp. Neur.*, LXXXII, 1945.
- Burmester, B. R., "A Study of the Physical and Chemical Changes of the Egg

- During its Passage Through the Isthmus and Uterus of the Hen's Oviduct,"
 — *Jour. Exp. Zool.*, LXXXIV, 1940.
- Chen, B. K., "The Early Development of the Duck's Egg, with Special Reference to the Origin of the Primitive Streak," *Jour. Morph.*, LIII, 1932.
- Cole, R. K., "Histology of the Oviduct of the Fowl in Relation to Variations in the Condition of the Firm Egg Albumen," *Anat. Rec.*, LXXI, 1938.
- Congdon, E. D. and Wang, H. W., "The Mechanical Processes Concerned in the Formation of the Differing Types of Aortic Arches of the Chick and the Pig and in the Divergent Early Development of their Pulmonary Arches," *Am. Jour. Anat.*, XXXVII, 1926.
- Conrad, R. M. and Phillips, R. E., "The formation of the Chalazae and Inner Thin White in the Hen's Egg," *Poultry Science*, XVII, 1938.
- , and Scott, H. M., "The formation of the Egg of the Domestic Fowl," *Physiol. Rev.*, XVIII, 1938.
- , and Warren, D. C., "The Alternate White and Yellow Layers of Yolk in Hen's Ova," *Poultry Science*, XVIII, 1939.
- Danchakoff, V., "Über das Auftreten der Blutelemente im Hühnerembryo," *Folia Haematologia*, IV, Suppl., 1907. — "Die erste Entstehung der Blutzellen beim Hühnerembryo und der Dottersack als blutbildendes Organ," *Anat. Hefte*, XXXVII, 1908a.
- Dudley, J., "The Development of the Ultimobranchial Body of the Fowl, *Gallus Domesticus*," *Am. Jour. Anat.*, LXXI, 1942.
- Duval, M., *Atlas d'embryologie*, Paris, 1889.
- Eastlick, H. L., "Studies on Transplanted Embryonic Limbs of the Chick. I. The Development of Muscle in Nerveless and in Innervated Grafts," *Jour. Exp. Zool.*, XCIII, 1943.
- Edwards, C. L., "The Physiological Zero and the Index of Development for the Egg of the Domestic Fowl, *Gallus Domesticus*," *Am. Jour. Physiol.*, VI, 1902.
- Evans, H. M., "On the Development of the Aortæ, Cardinal and Umbilical Veins, and other Blood Vessels of Vertebrate Embryos from Capillaries," *Anat. Rec.*, III, 1909.
- Firket, Jean, "On the Origin of Germ Cells in Higher Vertebrates," *Anat. Rec.*, XVIII, 1920.
- Foster, M., and Balfour, F. M., *The Elements of Embryology* (2 ed.), London, 1883.
- Fraps, R. M., Neher, B. H., and Rothchild, I., "The Imposition of Diurnal Ovulatory and Temperature Rhythms by Periodic Feeding of Hens Maintained under Continuous Light," *Endocrinology*, XL, 1947.
- Gasser, E., *Beiträge zur Entwicklungsgeschichte der Allantois, Müllerschen Gänge und des Afters*, Frankfurt a M., 1893. — "Die Entstehung der Cloakenöffnung bei Hühnerembryonen," *Arch. Anat. u. Entw.*, 1880.
- Goldsmith, J. B., "The History of the Germ Cells in the Domestic Fowl," *Jour. Morph. and Physiol.*, XLVI, 1928. — "The Primordial Germ Cells of the Chick. I. The Effect on the Gonad of Complete and Partial Removal of the 'Germinal Crescent' and of Removal of Other Parts of the Blastodisc," *Jour. Morph.*, LVIII, 1935.
- Greil, A., "Beiträge zur vergleichenden Anatomie und Entwicklungsgeschichte des Herzens und des Truncus arteriosus der Wirbelthiere," *Morph. Jahrb.*, XXXI, 1903.
- Gruenwald, P., "Normal and Abnormal Detachment of Body and Gut from the Blastoderm in the Chick Embryo, with Remarks on the Early Development of the Allantois," *Jour. Morph.*, LXIX, 1941.
- Guyer, M., "The Spermatogenesis of the Domestic Chicken (*Gallus domesticus*)," *Anat. Anz.*, XXXIV, 1909.

- Hamburger, V., "Morphogenetic and Axial Self-differentiation of Transplanted Limb Primordia of 2-day Chick Embryos," *Jour. Exp. Zool.*, LXXVII, 1938. — "The Development and Innervation of Transplanted Limb Primordia of Chick Embryos," *Jour. Exp. Zool.*, LXXX, 1939. — "The Effects of Peripheral Factors on the Proliferation and Differentiation in the Spinal Cord of Chick Embryos," *Jour. Exp. Zool.*, XCVI, 1944.
- Harman, M. T., "Concerning the Origin of the Notochord in the Chick," *Anat. Rec.*, XXIII, 1922.
- Hertwig, O. (Editor), *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere*, Jena, 1906.
- Hill, C., "Developmental History of Primary Segments of the Vertebrate Head," *Zool. Jahrb.*, XIII, 1900.
- Hillemann, H. H., "An Experimental Study of the Development of the Pituitary Gland in Chick Embryos," *Jour. Exp. Zool.*, XCIII, 1943.
- Hirota, S., "On the Sero-Amniotic Connection and the Fœtal Membranes in the Chick," *Jour. Univ. Tokyo*, VI, 1894.
- d'Hollander, F.-G., "Recherches sur l'oogenèse et sur la structure et la signification du noyau vitellin de Balbiani chez les Oiseaux," *Arch. d'Anat. Micr.*, VII, 1904.
- Hunt, E. A., "The Differentiation of Chick Limb Buds in Chorio-allantoic Grafts, with Special Reference to the Muscles," *Jour. Exp. Zool.*, LXII, 1932.
- Hunt, T. E., "The Development of Gut and Its Derivatives from the Mesectoderm and Mesentoderm of Early Chick Blastoderms," *Anat. Rec.*, LXVIII, 1937. — "The Origin of Entodermal Cells from the Primitive Streak of the Chick Embryo," *Anat. Rec.*, LXVIII, 1937.
- Jacobson, W., "The Early Development of the Avian Embryo. I. Endoderm Formation," *Jour. Morph.*, LXII, 1938. — "II. Mesoderm Formation and the Distribution of Presumptive Embryonic Material," *Jour. Morph.*, LXII, 1938.
- Jones, D. S., "The Origin of the Sympathetic Trunks in the Chick Embryo," *Anat. Rec.*, LXX, 1937. — "Studies on the Origin of Sheath Cells and Sympathetic Ganglia in the Chick," *Anat. Rec.*, LXXIII, 1939. — "Further Studies on the Origin of Sympathetic Ganglia in the Chick Embryo," *Anat. Rec.*, LXXIX, 1941. — "The Origin of the Vagi and the Parasympathetic Ganglion Cells of the Viscera of the Chick," *Anat. Rec.*, LXXXII, 1942.
- Kaupp, B. F., *The Anatomy of the Domestic Fowl*, Philadelphia and London, 1918.
- Keibel, F., and Abraham, K., *Normaltafeln zur Entwicklungsgeschichte des Huhnes (Gallus domesticus)*, Jena, 1900.
- Kellicott, W. E., *Outlines of Chordate Development*, New York, 1913.
- Kellogg, H. B., "The Course of the Blood Flow through the Fœtal Mammalian Heart," *Am. Jour. Anat.*, XLII, 1928.
- Kennedy, J. A. and Clark, S. L., "Observations on the Ductus Arteriosus of the Guinea Pig in Relation to its Method of Closure," *Anat. Rec.*, LXXIX, 1941.
- Kopsch, F., "Ueber die Bedeutung des Primitivstreifens beim Hühnerembryo und über die ihm homologen Teile bei den Embryonen der niederen Wirbeltiere," *Intern. Monatschr.*, XIX, 1902.
- Kuo, Z. Y., "Ontogeny of Embryonic Behavior in Aves. I. The Chronology and General Nature of the Behavior of the Chick Embryo," *Jour. Exp. Zool.*, LXI, 1932. — "II. The Mechanical Factors in the Various Stages Leading to Hatching," *Jour. Exp. Zool.*, LXII, 1932.
- Lillie, F. R., *The Development of the Chick*, 2 ed., New York, 1919.
- Locy, W. A. and Larsell, O., "The Embryology of the Bird's Lung Based on Observations of the Domestic Fowl," Part II, *Am. Jour. Anat.*, XX, 1916.
- Marshall, A. M., *Vertebrate Embryology*, New York and London, 1893.

- Martindale, F. M., "Initiation and Early Development of Thyrotropic Function in the Incubating Chick," *Anat. Rec.*, LXXIX, 1941.
- Morgan, T. H., *Experimental Embryology*, New York, 1927.
- Munro, S. F., "Functional Changes in the Fowl Sperm during their Passage through the Excurrent Ducts of the Male," *Jour. Exp. Zool.*, LXXIX, 1938.
- Murray, P. D. F., "Chorio-Allantoic Grafts of Fragments of the Two-Day Chick, with Special Reference to the Development of the Limbs, Intestine, and Skin," *Austral. J. Exp. Biol. and Med. Sci.*, IV, 1928.
- Olsen, M. W., "Maturation, Fertilization, and Early Cleavage in the Hen's Egg," *Jour. Morph.*, LXX, 1942.
- Pasteels, J., "Etudes sur la Gastrulation des Vertébrés Méroblastiques. III. Oiseaux. IV. Conclusions générales," *Arch. Biol.*, XLVIII, 1937. — "On the Formation of the Primary Entoderm of the Duck (*Anas Domestic*) and on the Significance of the Bilaminar Embryo in Birds," *Anat. Rec.*, XCIII, 1945.
- Patten, B. M., *The Early Embryology of the Chick*, 3 ed., Philadelphia, 1929. — "The Closure of the Foramen Ovale," *Am. Jour. Anat.*, XLVIII, 1931.
- , and Kramer, T. C., "The Initiation of Contraction in the Embryonic Chick Heart," *Am. Jour. Anat.*, LIII, 1933.
- , Sommerfield, W. A. and Paff, G. H., "Functional Limitations of the Foramen Ovale in the Human Fetal Heart," *Anat. Rec.*, XLIV, 1929.
- Patterson, J. T., "The Order of Appearance of the Anterior Somites in the Chick," *Biol. Bull.*, XIII, 1907. — "On Gastrulation and the Origin of the Primitive Streak in the Pigeon's Egg: Preliminary Notice," *Biol. Bull.*, XIII, 1907. — "Gastrulation in the Pigeon's Egg: A Morphological and Experimental Study," *Jour. Morph.*, XX, 1909. — "An Experimental Study on the Development of the Vascular Area of the Chick Blastoderm," *Biol. Bull.*, XVI, 1909. — "Studies on the Early Development of the Hen's Egg: I. History of the Early Cleavage and of the Accessory Cleavage," *Jour. Morph.*, XXI, 1910.
- Pearl, R., "Studies on the Physiology of Reproduction in the Domestic Fowl: I. Regulation of the Morphogenetic Activity of the Oviduct," *Jour. Exp. Zool.*, VI, 1909. II. (With Curtis, M. R.) "Data regarding the Physiology of the Oviduct," *Jour. Exp. Zool.*, XII, 1912.
- Peebles, F., "The Location of the Chick Embryo upon the Blastoderm," *Jour. Exp. Zool.*, I, 1904.
- Peter, K., "Untersuchungen über die Entwicklung des Dotterentoderms. I. Die Entwicklung des Entoderms beim Hühnchen," *Zeit. mikr. Anat. Forsch.*, XLIII, 1938. — "II. Die Entwicklung des Entoderms bei der Taube," *Zeit. mikr. Anat. Forsch.*, XLIII, 1938.
- Pohlman, A., "The Course of the Blood through the Heart of the Fetal Mammal with a Note on the Reptilian and Amphibian Circulations," *Anat. Rec.*, III, 1909.
- Popoff, D., *Die Dottersack-Gefäße des Huhnes*, Wiesbaden, 1894.
- Quiring, D. P., "The Development of the Sino-atrial Region of the Chick Heart," *Jour. Morph.*, LV, 1933.
- Rawles, M. E., "A Study in the Localization of Organ-forming Areas in the Chick Blastoderm of the Head-process Stage," *Jour. Exp. Zool.*, LXXII, 1936.
- Remak, R., *Untersuchungen über die Entwicklung der Wirbelthiere*, Berlin, 1855.
- Riddle, O., "On the Formation, Significance, and Chemistry of the White and Yellow Yolk of Ova," *Jour. Morph.*, XXII, 1911.
- Röthig, P. and Brugsch, T., "Die Entwicklung des Labyrinths beim Huhn," *Arch. mikr. Anat.*, LIX, 1902.
- Rudnick, D., "Differentiation in Culture of Pieces of the Early Chick Blastoderm. I. The Definitive Primitive Streak and Head-process Stages," *Anat. Rec.*, LXX,

1938. — "Contributions to the Problem of Neurogenic Potency in Post-nodal Isolates from Chick Blastoderms," *Jour. Exp. Zool.*, LXXVIII, 1938. — "Differentiation in Culture of Pieces of the Early Chick Blastoderm. II. Short Primitive Streak Stages," *Jour. Exp. Zool.*, LXXXIX, 1938. — "Early History and Mechanics of the Chick Blastoderm," *Quart. Rev. Biol.*, XIX, 1944.
- Scott, H. M. and Huang, Wai-Lan, "Histological Observations on the Formation of the Chalaza in the Hen's Egg," *Poultry Science*, XX, 1941.
- Scott, H. M. and Warren, D. C., "Influence of Ovulation Rate on the Tendency of the Fowl to Produce Eggs in Clutches," *Poultry Science*, XV, 1936.
- Spratt, N. T., "Location of Organ Specific Regions and Their Relationship to the Development of the Primitive Streak in the Early Chick Blastoderm," *Jour. Exp. Zool.*, LXXXIX, 1942. — "Formation of the Primitive Streak in the Explanted Chick Blastoderm Marked with Carbon Particles," *Jour. Exp. Zool.*, CIII, 1946. — "Regression and Shortening of the Primitive Streak in the Explanted Chick Blastoderm," *Jour. Exp. Zool.*, CIV, 1947.
- Stanley, A. J. and Witschi, E., "Germ Cell Migration in Relation to Asymmetry in the Sex Glands of Hawks," *Anat. Rec.*, LXXVI, 1940.
- Swift, C. H., "Origin and Early History of the Primordial Germ Cells in the Chick," *Am. Jour. Anat.*, XV, 1914.
- Verdun, M. P., "Sur les dérivés branchiaux du Poulet," *C. R. Soc. Biol. Paris*, V, 1898.
- Warren, D. C. and Scott, H. M., "Influence of Light on Ovulation in the Fowl," *Jour. Exp. Zool.*, LXXIV, 1936.
- Waters, N. F., "Changes in the Position of Chick Embryos after the Eighteenth Day of Incubation," *Science*, LXXXII, July 19th, 1935.
- Wetzel, R., "Untersuchungen am Hühnchen. Die Entwicklung des Keims während der erste beiden Bruttage," *Arch. Entw.-mech.*, CXIX, 1929.
- Whitehead, W. H., "A Working Model of the Crossing Caval Blood Streams in the Fetal Right Atrium," *Anat. Rec.*, LXXXII, 1942.
- Williams, L. W., "The Somites of the Chick," *Am. Jour. Anat.*, XI, 1910.
- Willier, B. H., "A Study of the Origin and Differentiation of the Suprarenal Gland in the Chick Embryo by Chorio-Allantoic Grafting," *Physiol. Zool.*, III, 1930.
- , and Rawles, M. E., "Developmental Relations of the Heart and Liver in Chorio-Allantoic Grafts of Whole Chick Blastoderms," *Anat. Rec.*, XLVIII, 1931.
- Windle W. F. and Becker, R. F., "The Course of the Blood through the Fetal Heart. An Experimental Study in the Cat and Guinea Pig," *Anat. Rec.*, LXXVII, 1940.
- Winiwarter, H. de, "Origine et Développement du Ganglion Carotidien. Appendice: Participation de l'hypoblaste à la Constitution des Ganglions Craniens," *Arch. Biol.*, L, 1939.
- Witschi, E., "Origin of Asymmetry in the Reproductive System of Birds," *Am. Jour. Anat.*, LVI, 1935.
- Woodside, G. L., "The Influence of the Host Age on Induction in the Chick Blastoderm," *Jour. Exp. Zool.*, LXXV, 1937.
- Young, R. T., "Origin of the Notochord in Chordates," *Anat. Rec.*, XXV, 1923.
- Yntema, C. L., "Experiments on the Origin of the Sensory Ganglia of the Facial Nerve in the Chick," *Jour. Comp. Neur.*, LXXXI, 1944.
- Zwilling, E., "Regulation in the Chick Allantois," *Jour. Exp. Zool.*, CI, 1946.

APPENDIX TO CHICK BIBLIOGRAPHY

- Brizzee, K. R., "Histogenesis of the supporting tissue in the spinal and the sympathetic trunk ganglia in the chick," *Jour. Comp. Neur.*, XCI, 1949.
- Cairns, J. M., "The influence of embryonic mesoderm on the regional specification of epidermal derivatives of the chick," *Jour. Exp. Zool.*, CXXXVII, 1954.
- Coughlin, F. E., Jr. and Walker, R., "Ductus arteriosi and their closure in the chick," *Anat. Rec. Absts.*, CXVII, 1953.
- Fraser, R. C., "Studies on the hypoblast of the young chick embryo," *Jour. Exp. Zool.*, CXXXVI, 1954.
- Gaertner, R. A., "Development of the posterior trunk and tail of the chick embryo," *Jour. Exp. Zool.*, CXI, 1949.
- Hamburger, V. and Hamilton, H. L., "A series of normal stages in the development of the chick embryo," *Jour. Morph.*, LXXXVIII, 1951.
- Hammond, W. S., "Origin of the thymus in the chick embryo," *Jour. Morph.*, XCV, 1954.
- Levi-Montalcini, R. and Amprino, R., "Recherches experimentales sur l'origine du ganglion ciliaire dans l'embryon de poulet," *Arch. de Biol.*, LVIII, 1947.
- Levi-Montalcini, R., "The origin and development of the visceral system in the spinal cord of the chick embryo," *Jour. Morph.*, LXXXVI, 1950.
- McKeehan, M. S., "A quantitative study of self differentiation of transplanted lens primordia in the chick," *Jour. Exp. Zool.*, CXXXVI, 1954.
- Olsen, M. W. and Fraps, R. M., "Maturation changes in the hen's ovum," *Jour. Exp. Zool.*, CXIV, 1950.
- Randles, C. A., Jr. and Romanoff, A. L., "Some physical aspects of the amnion and allantois of the developing chick embryo," *Jour. Exp. Zool.*, CXVI, 1950.
- Straus, W. L., Jr. and Rawles, M. E., "An experimental study of the origin of the trunk musculature and ribs in the chick," *Am. Jour. Anat.*, XCII, 1953.
- Waterson, R. L., Fowler, I. and Fowler, B. J., "The role of the neural tube and notochord in development of the axial skeleton of the chick," *Am. Jour. Anat.*, XCV, 1954.
- Yntema, C. L. and Hammond, W. S., "The origin of intrinsic ganglia of trunk viscera from vagal neural crest in the chick embryo," *Jour. Comp. Neur.*, CI, 1954. — "Experiments on the origin and development of the sacral autonomic nerves in the chick embryo," *Jour. Exp. Zool.*, CXXXIX, 1955.

PART V

THE MAMMAL

T

HE EARLY DEVELOPMENT OF THE MAMMAL AND ITS EMBRYONIC APPENDAGES

INTRODUCTION

IN taking up the development of the Mammal in a book of this type, intended primarily for college undergraduates, the writer faces a dilemma in the choice of material. For those interested chiefly in Zoology the comparative aspects of early stages in several selected Mammals, suggesting as they do evolutionary trends, are highly significant. On the other hand for those mainly intent upon the study of medicine the emphasis of interest is likely to be different. Such students, and many of their teachers, though willing to admit that the study of early comparative mammalian development is of some value, feel that for practical purposes they must begin to concentrate. Hence they prefer to consider chiefly the embryology, both early and later, of a single form. Preferably this would be Man, but since that is usually not practical, the next best thing is to select for study some readily available Mammal whose history is nearly akin to that of Man. That Mammal is generally the Pig. If space allowed, there is of course no reason why both these lines could not be followed in considerable detail. Unfortunately, however, in a book already dealing at some length with the Frog and Chick, space does not permit an extensive treatment of both topics. Consequently the following compromise way of treating the Mammals becomes necessary.

To begin with, it will be found desirable as in previous cases to go back of the start of the embryo itself, and consider somewhat the reproductive organs of the adults. This will be especially necessary in the mammalian females because of the special relation of certain of their organs to the reproductive process and to the developing young.

We shall then proceed with the comparisons of the early embryos of selected orders of Mammals with special emphasis upon the develop-

ment and character of their extra-embryonic membranes and structures. This special emphasis is pertinent because we shall find that these membranes and organs are fundamentally similar to those already familiar in the Chick, and found in all Sauropsids, i.e., Birds and Reptiles. They are of present interest because of the manner in which both their origin and structure has been modified in the different mammalian groups to serve essentially their old functions. The modifications have resulted from the different environment in which the embryo and fetus of the Mammal occurs, and from the very special relations with the mother which this environment makes necessary. That there should be similarities in these structures as between the Mammals and the Sauropsids is of course natural in view of the known derivation of the Mammals from the Reptiles. The modifications in the mammalian orders selected then help to suggest the lines along which evolution has perhaps moved within that class.

Having thus compared the early stages of certain representative mammalian forms, we shall finally concentrate upon one of them, i.e., the development of the Pig. The Pig, however, is an Ungulate, and the Ungulates are one of the groups whose earliest stages and extra-embryonic membranes have been chosen for comparative study. In this latter study, moreover, the Pig will be especially emphasized as an example of the group. Hence when we come to the detailed consideration of this animal it will not be necessary to start quite at the beginning. We shall simply pick up where the comparative account left off.

Lastly, another device by which we shall endeavor to save space and time is the following: In the embryology of the Frog and Chick we have already twice gone over in some detail the development of all the main vertebrate systems. In the Chick, moreover, the processes in many cases are, as has already been suggested, very similar indeed to those found in the Mammal. Hence in the Pig we shall not repeat again in detail the development of each system. Instead we shall outline such development rather briefly, emphasizing only those points in which the process or structure in this animal significantly differs from that in the Chick. Such treatment will of course be accompanied by as many illustrations as possible. This should be sufficient, and will be so if the student of the Pig has reasonably well in mind the corresponding situations in the Chick. Anyone who does not have the Chick development clearly in mind will find it necessary to refresh the memory by reference back to the appropriate account in that form.

THE REPRODUCTIVE ORGANS OF THE ADULT

THE MALE

The Testes and Their Ducts. — In the adult male Mammal there are normally two testes. These organs may be retained permanently within the body of the animal, as in the case of the Elephant; more commonly, however, they pass out of the body during development, and are contained either in two sacs, or in two chambers of a single one, the *scrotal sac* or *scrotum*. This is the case in the Pig. In some cases, however, as among Rodents, an intermediate condition occurs in which the testes descend into the scrotum only during intervals of sexual activity. Each testis consists of the usual seminiferous tubules, embedded in connective tissue and leading by way of vasa efferentia to the respective vas deferens.

Accessory Organs. — In the Mammal there are, in addition to the testes and other parts just noted, certain accessory organs connected with the more distal parts of the genital tract. These are the *prostate glands*, *Cowper's glands*, and, in some animals (e.g., in the Pig and in Man), the *seminal vesicles*. The function of the glands is to furnish a suitable medium for the existence of the sperm after it leaves the organs of the male. The vesicles presumably assist both in the secretion of additional fluids and in storing the combined sexual products or *semen* previous to its ejaculation. Finally, there is in the male Mammal a *penis*. This has a single duct, the *urethra*, which serves to discharge urine, and also to introduce the semen into the genital tract of the female.

THE FEMALE

The Ovary. — In the female Mammal there is a single pair of ovaries, and, as in the other forms studied, these organs are contained within the body cavity and suspended from its wall by a mesovarium. The ovaries are whitish ovoid objects, varying in size in different animals, but always relatively small. Thus in the Human Being, for example, each ovary is about 3–4 cm. long, and from 2–3 cm. wide, and they are about the same in the Pig. Fundamentally, their internal structure is similar to that already described in the Bird.

The Genital Tract.

The Oviducts. — As in the Bird, the ovaries are not directly connected with the Müllerian ducts or oviducts. The latter, sometimes

known as the *Fallopian tubes*, are, however, provided as usual with a typical fimbriated funnel, or *infundibulum*, which serves to embrace the ovary when an ovum is discharged. The walls of the oviducts are made up as follows: On the outside is the *serous membrane*, next to that a layer of more or less mingled *longitudinal and circular muscles*, then a sheet of vascular connective tissue covered by ciliated epithelium. the connective tissue with its epithelium being known as the *mucous layer*.

From each infundibulum the respective duct proceeds to join the one from the opposite side. Between the infundibulum and the point of junction, however, there is usually more or less bending, and in many cases the duct actually starts anteriorly before curving backward and medially to unite with its fellow.

The Uterus and Vagina. — At some point distal to the infundibula either above or below the region of junction, or in some cases both above and below, the character of the tract or tracts changes. The muscular wall becomes thicker as does also the mucous layer which now contains lymph spaces and many glands. The part or parts of the genital tract thus characterized are then known as the *uterus* or *uteri*, and the thickened mucous layer plus its epithelium are referred to together as the *uterine endometrium*. When these changes occur entirely proximal to the point of union of the tubes so that there are two distinct uteri (Rodents) the condition is known as *uterus duplex*. On the other hand when they occur both above and below the region of union (Carnivores and Ungulates) the situation is described as *uterus bicornis*. Finally, when the uterine character exists only in the fused part of the tract the condition is called *uterus simplex*.

Beyond the uterus, or uteri, as the case may be, there is a single passage leading to the exterior, known as the *vagina*. At the external end of the latter there are certain rudiments homologous with the penis of the male.

THE DEVELOPMENT OF THE OVUM UP TO SEGMENTATION, AND THE SEXUAL CYCLE

OÖGENESIS

The Oögonia. — The embryonic ovary of the Mammal contains the usual primordial germ cells which, as in the lower Vertebrates, have probably migrated thither from the walls of the gut. At first these cells lie chiefly in the outer epithelium or cortex of the ovary. According to

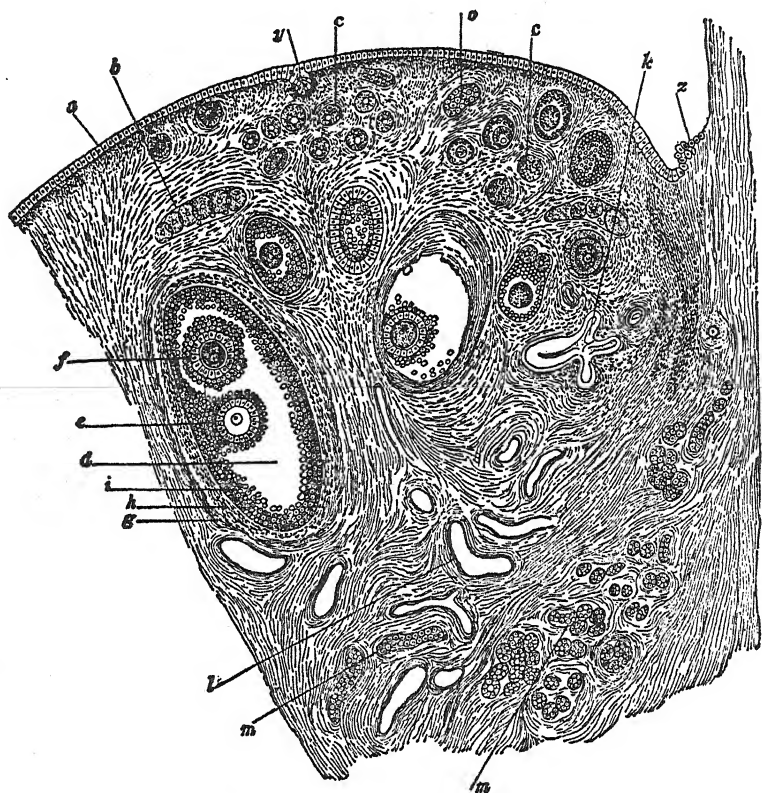


Fig. 247. — Section through part of the ovary of a Dog. From Kellicott (*Chordate Development*). After Waldeyer.

a. "Germinal epithelium." b. Ovigerous cords. c. Small ovarian follicles. d. Older ovarian follicle. e. Ovum surrounded and attached to wall of follicle by cells of discus proligerus (cumulus oöphorus), including those of the future corona radiata. f. Second ovum in follicle with e. (Only rarely are two ova thus found in a single follicle.) g. Outer layer of follicular capsule. h. Inner layer of follicular capsule. i. Membrana granulosa. k. Collapsed, degenerating follicle. l. Blood-vessels. m. Sections through tubes of the parovarium. y. Involved portion of superficial epithelium. z. Transition to peritoneal epithelium.

most accounts this cortical epithelium thickens and then produces outgrowths which push into the deeper mesenchyme. These outgrowths are the *ovigerous cords* similar to those described in the Chick, but in this instance often called the *cords of Pflüger*.¹ As in the Bird, they contain both the female germ cells, or oögonia, and numerous epithelial cells as

¹ Also according to some recent studies by Gruenwald ('42) the development of the cords is somewhat more involved than this, and varies to some extent in different Mammals. The end result, however, is essentially as indicated.

well. In the Mammal, however, the two types of cells are not easily distinguishable from one another, and it is quite possible that some germ cells may arise *in situ* from indifferent cells of Pflüger. During this period multiplication of all the cells goes on rapidly.

At some time before the birth of the animal in which the ovary is contained the multiplication of the oögonia is said to cease. As has been previously noted, however, this assertion is now seriously questioned, some workers (E. Allen, '23, G. I. Hargitt, '30, and others) maintaining that in certain cases at least the ova derived from the primordial germ cells all, or nearly all, disappear. These are then said to be replaced by new oögonia arising from the peritoneal (germinal?) epithelium at intervals during the sexual life of the individual. In any event the cells are eventually arranged in nests or groups, each of which contains a single oögonium, the remaining epithelial cells in the group being destined to form the follicle. The young ovum now enters upon the growth period as an oöcyte.

The Oöcyte and the Graafian Follicle. — At about this time, the epithelial cells referred to begin to become arranged about the young ovum to form the highly characteristic mammalian or *Graafian follicle*. At first they constitute a thin flat layer only one cell thick, but soon multiply so as to form a mass of cells about the growing oöcyte. In one side of this mass there then appears a space, the *follicular cavity*, which gradually enlarges and extends around the sides of the oöcyte. These extensions, however, never quite meet. Thus the oöcyte, still closely surrounded by several layers of cells, is suspended within the follicular cavity, which becomes filled by a fluid, the *liquor folliculi*. Meantime, the outside of the entire follicle has become covered by a capsule (*follicular capsule* or *theca*), formed externally of connective tissue (*theca externa*) and internally of cells, blood vessels, and nerves (*theca interna*).

The various layers and parts of the entire Graafian follicle may now be named, as follows: Beginning on the outside there is the follicular capsule (theca) with its inner and outer layer. Just within this, and bounding the follicular cavity, there are a few layers of the follicular cells forming the *basement membrane*, or *membrana granulosa*. Upon the side of the ovum where the cavity has not extended, a neck of cells reaches from this membrane to those cells which immediately surround the oöcyte. Thus the latter is attached to the inner wall of the follicle by this neck, which, together with the more peripheral of the cells immediately surrounding the ovum, is termed the *discus proligerus* or

cumulus oöphorus. Those of the immediately surrounding cells which have remained closest about the egg are now gradually elongated at right angles to the surface of the latter. Many of these cells remain attached to this surface for a time following ovulation when they become known as the *corona radiata* (Figs. 247, 248). This brings us to the actual egg and its membrane.

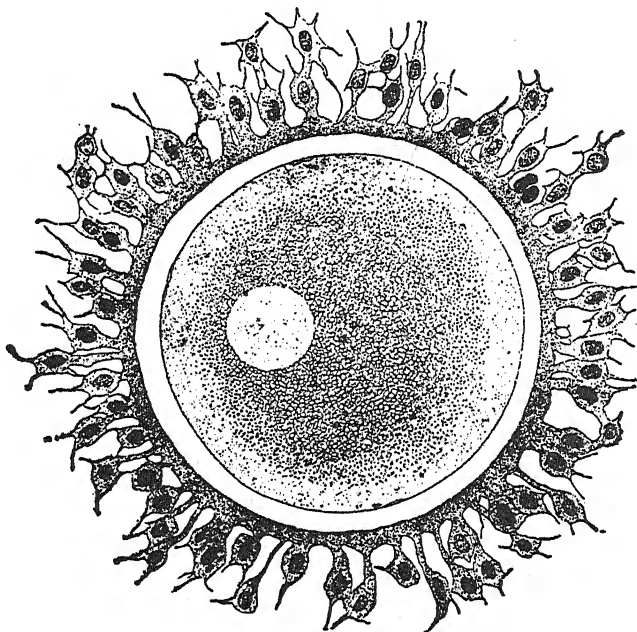


Fig. 248. — Fully grown Human oöcyte just removed from the ovary. Outside the oöcyte are the clear zona pellucida and the follicular epithelium (*corona radiata*). The perivitelline space in this instance is not apparent. The central part of the oöcyte contains deutoplasmic bodies and the excentric nucleus (germinal vesicle). Superficially there is a well-marked exoplasm, or cortical layer. From Waldeyer (Hertwig's *Handbuch*, etc.).

THE MATURE OVUM AND OVULATION

The Mature Ovum. — The mature ovum in all placental Mammals² is relatively minute, though naturally varying in size in different animals. Thus that of the Mouse measures about .075 mm. in diameter,

² It will suffice to state at this point that the term *placental Mammal* includes the vast majority of the group. Its exact significance will be fully described in the section on the yolk-sac, allantois and placenta (see below).

that of the Dog about 0.14 mm., that of Man 0.135 mm., and that of the Whale 0.14 mm. (Hartman, '29, '30). The reason for this minute size is the fact that mammalian eggs are virtually without yolk (alecithal). They consist of a central region of opaque endoplasm surrounded by a thin layer of exoplasm, and within the former is a relatively large nucleus (germinal vesicle), somewhat excentrically placed.

The ovum apparently does not possess any true vitelline membrane. It is surrounded, however, by a thick transparent substance which is presumably chorionic, i.e., is secreted by the cells of the follicle. This layer, though clear, frequently appears to be perforated by minute canals through which processes of the follicular cells reach the egg to nourish it. It is, therefore, known either as the *zona pellucida* or the *zona radiata*. There is usually a slight space between this zone and the protoplasm of the egg, and though there may be no vitelline membrane this space is known as the *perivitelline space* (Fig. 248).

Ovulation. — As a Graafian follicle and its ovum matures, it is gradually brought to the surface of the ovary. At the same time one side of the follicle becomes thin in connection with the formation of a *cicatrix*, as in the Chick. As complete maturity is reached, the discus proligerus is broken and the ovum floats freely in the liquor folliculi. In most animals rupture of the follicle then occurs spontaneously, and its contents is received by the infundibulum of the oviduct. In a few forms, e.g., the Rabbit and Cat, the breaking of the ripe follicle does not usually occur spontaneously, but only following copulation with the male (*coitus*). The liberation of an ovum may or may not take place in both ovaries at once, and there may or may not be more than one follicle ready for discharge in the same ovary at approximately the same time. These variations, moreover, may occur normally in the same species of animal. In Mammals which ordinarily produce a litter of young, however, the discharge of several ova at once is of course the usual thing.

THE SEXUAL CYCLE IN THE FEMALE

It is well known that like many other animals, Mammals are capable of breeding only during certain periods or seasons. Among this group, moreover, these periods are far more marked in the female than in the male. In the former sex they are also very definitely related to the process of ovulation so that it seems desirable to discuss the subject at this point. In all placental Mammals which have been carefully studied, it is known that during sexual life the walls of the uterus suffer a series of periodic changes, interrupted only by pregnancy. The placentals, more-

over, may be divided into two main groups with respect to these uterine changes, i.e., the Primates and the non-Primates.

The Non-Primate Cycle. — Among this group the stages involved are fundamentally similar, and these stages are well represented in the Pig, whose embryology will later be considered. We shall begin therefore by a description of the sexual cycle in the female of this animal. In the sow each sexual or *oestrus cycle*, as it is called, occupies twenty-one days and in the absence of pregnancy, the cycles are continuous throughout the year. As regards the behavior of the animal, the activity of the ovary, and the condition of the uterine endometrium, the periods or phases of a cycle are characterized as follows:

I. The Dioestrus. — During this period lasting about two and one half weeks the sow occupies herself with eating and sleeping, and shows no interest in the opposite sex. A study of her ovaries, however, shows that within this interval an important event takes place. The empty follicles which remain from the immediately preceding ovulation become filled with a specialized type of fatty cell. In some cases (Man) these cells are yellow in color, which has caused each body so formed to be known as a *corpus luteum*. In the Pig, however, these bodies are pinkish. They quickly develop to a maximum extent, and persist in this condition for about the first thirteen to fourteen days of the period, at which time they begin to regress. Correlated with the time of development and persistence of the corpora lutea in the ovary, the uterine mucosa, which was already quite thick at the beginning of this period, becomes even more hypertrophied, especially the glands. This is a condition known as *pseudopregnancy*, because, as we shall see, the state of the mucosa at this time resembles to a considerable degree its character during true pregnancy, and due to the stimulus of the same hormone, progesterone (see below). Finally as the corpora lutea regress the uterine mucosa likewise regresses, and within two or three days has become relatively thin (Fig. 249, A). Thus during the last day or so of the dioestrus there is virtually nothing going on in the uterus so that this brief interval may be thought of as a time of more or less complete "rest" for that organ.

II. The Pro-oestrus. — Following the dioestrus there is a short interval of a day or so generally known as the pro-oestrus, within which the behavior of the animal remains about as before. Studies of her ovaries, however, reveal that undeveloped Graafian follicles are starting a rapid growth, while the uterine mucosa also has again begun to hypertrophy (Fig. 249, A)

III. The Oestrus. — This period, lasting approximately three days, is known as the time of "heat," and during it the sow becomes extremely restless and will accept mating at any time. Examination of the ovaries shows that the Graafian follicles come to maturity at about the middle

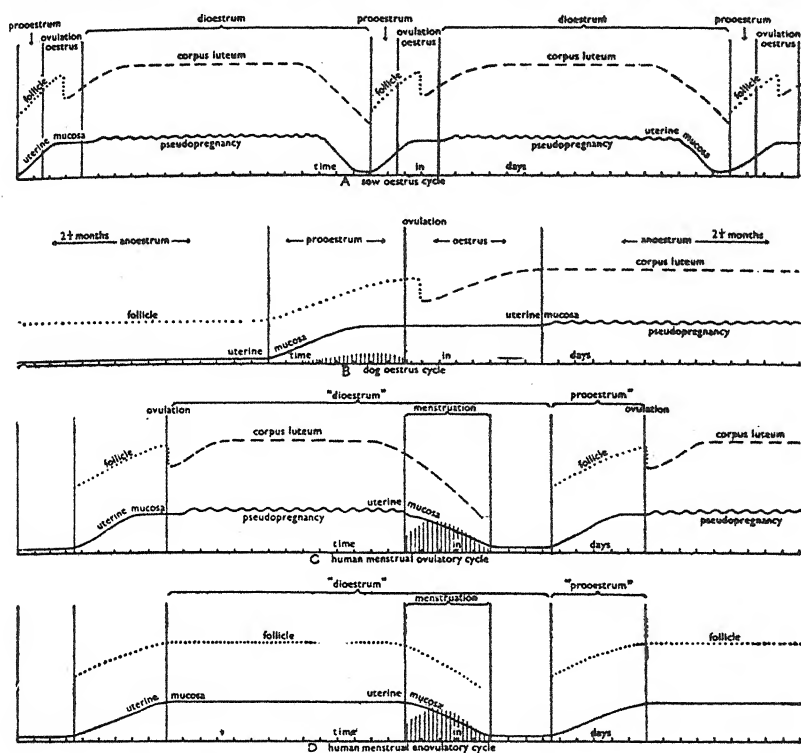


Fig. 249. — Diagrams comparing the events of the oestrus cycles of the Pig and Dog with those of the ovulatory and non-ovulatory menstrual cycle in Man. The fine vertical rulings in the cycle of the Dog and in those of Man indicate the time of occurrence and the approximate relative degree of bleeding in each case. There is no normal obvious bleeding in the Pig. The rise and fall of the curved lines indicates the relative degree of hypertrophy or degeneration of the tissues or bodies designated.

of this period, and at that point ovulation occurs. The corpora lutea, already referred to, immediately start development which, in the absence of pregnancy, continues into the succeeding dioestrus as already described. The hypertrophy of the mucosa, well under way at the end of the pro-oestrus, also continues on through oestrus and into the succeeding dioestrus, during most of which periods it remains at a high level as indicated (Fig. 249, A).

Variations in the Non-Primate Cycle.—The non-Primate cycle as thus described for the Pig may be considered typical for the non-Primate group of animals so far as its fundamental aspects are concerned. As already suggested, however, there are numerous variations in detail, some of the more striking of which will now be noted. Probably the most outstanding is that which occurs in animals like the Dog and Cat. In these animals there are only two or three oestrus periods a year, with a long inactive interval, known as an *anoestrus* between each period of "heat." In such cases the corpora lutea, and the uterine hypertrophy in the absence of pregnancy, only persist for a relatively short time, the uterine mucosa being comparatively thin during most of the long anoestrus. Breeding of course can only occur during the oestrus periods which are hence referred to as the *breeding seasons*. The Dog and Cow are further peculiar in that at the end of the pro-oestrus the blood vessels of the hypertrophied mucosa are so gorged that some superficial bleeding occurs. This quirk led to much discussion and misapprehension of the relations between the non-Primate and Primate cycles as we shall presently see. Another peculiarity of a few animals such as the Cat and also the Rabbit, as already noted, is the fact that ovulation in these forms is not spontaneous during oestrus, even though the mature ova are present. It only occurs at this time if copulation, or some form of stimulation which simulates copulation, takes place. Otherwise the ripe follicles simply degenerate, no corpora lutea are formed, and hence no pseudopregnancy occurs (see below).

Not only do animals vary as between those with a succession of relatively short dioestrus cycles like the Pig, and those with long anoestrus intervals like the Dog (Fig. 249, *B*), but in the latter type some forms have several short dioestrus cycles between each anoestrus. That is they have a breeding season perhaps once a year like some sheep, and during that season they come into "heat" several times. Animals with only one oestrus period at a breeding season are said to be *monoestrus*, while those with several at each season, or with continuous short cycles, are *polyoestrus*. Lastly the length of the dioestrus cycles varies greatly among different animals. Thus, while it is twenty-one days in the Pig, it is only five days in the Rat and Mouse, and fifteen in the Guinea-Pig. It should be emphasized also that these are average times. There is commonly some variation in cycle length even in the same individual, depending upon temperature, food and other unknown conditions.

The Primate Cycle.—In discussing this group it should at once be pointed out that the peculiarities about to be described do not actu-

ally apply to all Primates, e.g., to Lemurs and to the New World Monkeys. They do, however, apply to the Anthropoid Apes, the Old World Monkeys and to Man. The most complete studies have been made on Man and Rhesus, an Old World Monkey, and we shall therefore consider the situation particularly as it applies to these forms, and first especially as it applies to Man.

The Menstrual Cycle. — The peculiar characteristic of the sex cycle as it occurs in the Human female is the inclusion within it of the phenomenon of *menstruation*, from which the whole cycle takes its name. The nature of this phenomenon, and its relation to the parts of the non-Primate cycle, in so far as it can at present be related to them, is as follows:

Keeping the Pig in mind as presenting a typical example of the situation in the non-Primates, we find that the first but least important difference between that animal and Man is in the length of the entire cycle. Thus in the Pig, as just noted, it is about twenty-one days, while in both Women and the Rhesus monkey it is normally twenty-eight days, with numerous more or less minor variations. Proceeding next to a comparison of the periods within the cycle, and starting with the one in Man presumably homologous with the dioestrus in the lower animals, we find conditions at that stage in the Human subject about the same as in the sow. That is to say there is no sexual urge at this time, the ovary contains a corpus luteum, and at the beginning the uterine mucosa is hypertrophied. This phase, comparable with the first and major (pseudo-pregnant) part of the dioestrus, lasts for about two weeks. At the end of this time, as in the lower forms, the corpus luteum disappears, and accompanying this the uterine epithelium regresses. In this instance, however, this regression instead of being relatively quiet and uneventful, is a rather violent affair involving a serious breakdown of the endometrium, both mucosa and epithelium. This is accompanied by a sloughing off of cells and considerable bleeding, and it is this process which comprises menstruation. Following this as in the Pig, comes a "rest" interval, in this instance, however, lasting four to five days and involving repair of the preceding damage, though the mucosa remains relatively thin. Menstruation plus this interval would therefore correspond to the end of the dioestrus in the Pig, except that in that animal the process of regression is much less violent. Hence the menstrual features are lacking, and no "repair" is required during the "rest" interval.

The next period should be that of the pro-oestrus, and apparently something essentially similar to this in the lower animals exists in

Man. As in the former case it apparently involves no accentuation of sex interest, the ovary contains a maturing Graafian follicle, and the uterine mucosa begins again to hypertrophy. This lasts five to six days.

Following the "pro-oestrus" the next period should be that of oestrus, but this is another respect in which the Primate cycle differs from that of the non-Primates. There is no oestrus. This means that there is no time in the cycle of greatly heightened sexual activity. Ovulation, which should occur sometime during oestrus, occurs at the end of what we are calling the "pro-oestrus," though the use of this and other terms relating to the oestrus cycle is obviously questionable in a cycle in which there is no oestrus. This is why the Primate cycle is commonly referred to as the menstrual cycle in correlation with its most outstanding characteristic. Following ovulation a corpus luteum of course exists, and in the absence of pregnancy a new "dioestrus" begins, culminating in another menstruation and "rest" interval (Fig. 249, C). From this account it will be evident that ovulation occurs about midway between menstruations, i.e., from the twelfth to the sixteenth day following the beginning of the last menstrual period (Corner, '43). From this it is clear that menstrual bleeding has nothing whatever to do, either in relative time of occurrence, or in character, with the minor bleeding of the pro-oestrus in an animal like the Dog, a phenomenon with which it was once confused. In this connection it should be noted that a slight pro-oestral bleeding also occurs in the Rhesus Monkey and occasionally in Women, in which cases it is known as *intermenstrual bleeding* or Hartman's sign, i.e., a sign of imminent ovulation.

To summarize a comparison of the two cycles, then, we may say this: In both there is what amounts to a "dioestrus" during which sexual activity is not evident. The ovary contains a corpus luteum during the first part of this period, and during this part the uterine mucosa is hypertrophied. Near the end in both cases the mucosa regresses, but in the Primate cycle the regression is much more thoroughgoing, and is termed menstruation. Finally a short quiescent interval ensues which in the Primates is occupied with uterine repair. In both cycles a "pro-oestrus" follows the "dioestrus" involving no change in sex activity, but the growth of a new Graafian follicle and renewed uterine hypertrophy. In the non-Primate cycle this is followed by oestrus or "heat" in the midst of which ovulation occurs. In the Primate cycle ovulation occurs at the end of what we have called, for the sake of comparison, the "pro-oestrus," and there is no oestrus. Instead the "dioestrus" immediately follows, and the cycle is complete.

Having thus described the oestrus and the menstrual cycles there remain the problems of their causes and functions. Much work has been done in this connection over a long period, but it is only within recent years that the pieces of the puzzle have begun to fall into some semblance of order. As will presently appear, however, there are even yet some pieces which are missing.

Causes of the Oestrus and Menstrual Cycles.—It is already evident that certain events in both the oestrus and menstrual cycles are closely correlated. Thus we have seen that when a follicle is developing in the ovary the uterine mucosa in either cycle is undergoing its pro-oestral hypertrophy. As the corpora lutea form it undergoes still further hypertrophy, and when these latter bodies start to disappear this mucosa regresses, either with or without extensive breakdown. Why is this? The answer is found in the fact that the developing follicle produces a hormone called *oestrone* (theelin) which causes the initial pro-oestral hypertrophy. It also of course causes the behavioral phenomenon of "heat" in most "lower" animals.³ As the corpora lutea form following ovulation they also produce one or more hormones, including some oestrone. The most prominent of these, however, is called *progesterone*, and this causes the still further uterine hypertrophy of the first part of the dioestrus. Both these hormones are sterols, have been obtained in pure crystalline form, and their action repeatedly demonstrated experimentally. The withdrawal of the progesterone as the corpora lutea begin to disappear would then explain both the dioestral regression and the menstrual breakdown of the mucosa previously built up. The follicular and luteal hormones produced in the proper order and then withdrawn would therefore seem to account satisfactorily and completely for both types of cycle. This would be true were it not for one curious fact. It was discovered (Corner, '23) that Rhesus monkeys, and probably more rarely Women, experience menstruation without ovulation, and hence in the absence of corpora lutea. The monkeys, it should be noted, have a breeding season (the winter months), and it is at the beginning and end of this season that these so-called anovulatory cycles occur. Women of course have no such season, and in them cycles of this character have been thought to occur most commonly in girls beginning to menstruate. It is now known, however, that such anovulatory cycles, otherwise apparently normal, occur in a certain percentage of women during their active sexual life. Indeed it has been proven that such women may only

³ Just what parts of the follicle are responsible for this hormone is not altogether certain, but probably either the theca interna or the granulosa or both.

actually ovulate two or three times a year in spite of seemingly normal menstrual periods, causing serious interference with fertility. In any event such cycles obviously upset the foregoing neat explanation of the entire phenomenon. Much work has been done in an effort to solve this problem, but no completely satisfactory answer has yet been arrived at. It is known for instance that in castrate animals an apparently normal cycle can be produced by the injection and sudden withdrawal, after a suitable interval, of oestrone alone. Yet in non-castrate animals extra doses of oestrone will not prevent the uterine breakdown. A little progesterone, however, will do so. Hence the latter substance seems clearly to have some important part in the cycles of normal ovulating animals, probably in the manner already described.

With these facts in mind two possible explanations of the anovulatory cycle may be briefly noted. One, considered by many the most probable, is that a certain amount of oestrone is necessary, first to build up, and then to maintain, the uterine endometrium in a state of preovulatory hypertrophy. This hypertrophy is of course not quite like that produced by progesterone, but is nevertheless considerable. The necessary oestrone for this is furnished by the partially developed follicle, which instead of going on to ovulate, persists for a time, periodically regresses, and is replaced by another. The regression of course produces a temporary lack of oestrone, and an anovulatory endometrial breakdown very similar to menstruation occurs (Fig. 249, *D*). The second possibility, suggested by Hisaw, is that the partially developed Graafian follicle produces not only oestrone, but a little progesterone as well. Then if, in the anovulatory cycle, the production of the progesterone for some reason, such as the regression of the follicle, declines, this may be enough to produce menstruation even in the absence of ovulation and the ensuing corpus luteum. There is a little suggestive evidence for this, but it is difficult to prove. So much for this part of the oestral cycle and menstrual mechanism.⁴

⁴ It may be added that these hormones also have several other significant effects not directly pertinent to the present discussion. Thus oestrone not only starts the hypertrophy of the mucosa in each cycle, but is necessary to bring the infantile uterus to a stage of development where progesterone can act on it. Also it controls the growth of the muscles of the pregnant uterus, first stimulating, and then checking, and causes cornification of the vagina of the Guinea Pig, thus revealing its presence in this animal. Lastly it stimulates development of the breasts to a condition where they can be acted on by the pituitary hormone, prolactin, but at the same time prevents milk flow until birth. Progesterone in addition to its effect on the uterine mucosa has a decidedly quieting action on the normal rhythmic contractions of the uterine muscles, and is said by some to cause relaxation of the pelvic

There still remains the question as to what sets off these cycles, i.e., what starts the follicles to developing, and what stops them. The answer to this appears to be found in that gland-of-all-work, the pituitary. The anterior lobe of this gland is known to produce, among other things, a follicle stimulating hormone (F.S.H.) which causes Graafian follicles to begin their growth. What then seems to happen is that when the growing follicle achieves a certain output of oestrone this acts in turn to suppress secretion by the pituitary. (There is some experimental evidence for this.) The follicle then ovulates, and its extensive oestrone production ceases, thus allowing the pituitary secretion to rise again, and so the cycle repeats itself.

Here again, however, a problem arises which has not been entirely satisfactorily answered. The scheme just presented works well enough for animals like the Pig or Man with continuous cycles, but what of those with an anoestrus? What causes the cycles to stop? We do not know. It has been suggested that during the anoestrus in such animals as the Dog or Cat the secretion of the pituitary and the ovarian follicle, exactly balance each other so that nothing happens. Perhaps so, but there is no proof of it. Also if this is true, what produces an unbalance, and starts off a new cycle?

Functions of the Female Cycle. — Thus far the oestrus and menstrual cycles have been considered without reference to the possible occurrence of pregnancy. As might be suspected, however, each cycle is in fact an invitation to, and a preparation for, this important event. In cases where oestrus occurs the behavior of the female is such as to permit and encourage mating at this time, and it is of course at just this point also that a ripe egg is released into the oviduct ready to be fertilized. In the menstrual cycle the same thing is true, except that here there appears to be no special sexual urge at the time of ovulation. Following this event in either case the egg is subject to fertilization in the upper end of the oviduct. If this occurs the egg becomes what amounts to a blastula in a manner to be described below, and after 3-4 days finds its way into the uterus. Here meanwhile the climax in the hypertrophy of the uterine mucosa is coming about. It now appears that this hypertrophy is just what is needed to insure the firm attachment of the developing egg to the uterine wall by a process known as implantation. This

ligaments of the Guinea Pig. Hisaw, however, has claimed a separate luteal hormone, relaxin, to be responsible for this. In some cases progesterone also acts as an accessory in aiding the oestrogens to prepare the breasts for final stimulation by prolactin.

process varies considerably in different animals, and will be discussed at some length later on. The point to be noted at the moment is that apparently the hypertrophy of the mucosa is a necessary preparation for it. As has been noted, if fertilization and implantation fail to occur, the hypertrophy regresses and a new cycle is initiated, with, as M. S. Gilbert so cleverly suggests in her book, *Biography of the Unborn*, "hope for better luck next time." On the other hand, if implantation does occur, the hypertrophy persists and in fact increases. Because of the similarity of this hypertrophy to that of the dioestrus, the latter, as previously noted, is frequently termed pseudopregnancy. This persistence of the hypertrophy when it is needed, and its disappearance when it is not needed leads to some further questions to which we have at present only partial answers. Some of these questions and the tentative answers are as follows:

What for instance makes the hypertrophy of the mucosa persist in pregnancy and not at other times? In this connection it is of interest to find that in many animals the corpora lutea also persist throughout pregnancy instead of disappearing as in the non pregnant cycle. Is there a causal connection here? It would appear that in those cases where both corpora lutea and mucosal hypertrophy persist together there is. Thus in the Rat and the Cow removal of the corpora lutea of pregnancy causes regression of the mucosa and abortion, though in other cases, like that of Man, this is not true. The answer as to what makes the hypertrophied uterine mucosa continue in the former animals then seems to be fairly clear. It will be recalled that one of the chief hormones of the corpus luteum is progesterone. This hormone, however, was so named because of the very fact that it maintains an hypertrophied condition of the mucosa not only during most of the dioestrus, but especially during pregnancy. Thus the corpora lutea apparently rather obviously persist during pregnancy in these cases in order to secrete the progesterone which maintains this condition. There is also, as noted, evidence that the corpora lutea produce some oestrone, or something closely akin to it. This and the progesterone appear to assist in causing the hypertrophy of the muscles of the uterus as well as that of the mucosa during pregnancy.

The next question is, how do the corpora lutea know, so to speak, when to persist and when not to? The answer to this appears to be that the organ which attaches the embryo to the uterine wall, termed the placenta, itself secretes several hormones, one of which is luteinizing, i.e., helps to keep the corpus luteum developed. There is also a pituitary hor-

none which has a luteinizing effect, but this is apparently not the one chiefly involved during pregnancy. As just suggested the placenta produces other hormones, i.e., oestrogens (oestrone like hormones), and also quite definitely progesterone. This source of these substances, it is now generally agreed, soon becomes the main one in cases like Man where the corpus luteum functions for only about the first four months of pregnancy, being operatively removable after the first few weeks without harm.

Also, in Man at least, certain other gonad stimulating hormones, similar in action to the F.S.H. of the pituitary, are produced by the placenta. They are called Prolan A and B, and are used in the Aschheim-Zondek or Friedman tests for pregnancy. Thus so much of these hormones is produced under this condition, even within the first month, that they are excreted in the urine. Advantage is taken of this fact to make a test for their presence, and hence for pregnancy, by injecting a specified amount of the suspected urine into a female rabbit (Friedman test). If the hormones are present they will cause the animal to ovulate within ten hours.⁵ The particular tissue of the placenta from which these various sterol substances appear to be derived in Man and Monkeys is a special material called trophoblast to be described below (Wislocki and Bennett, '43; Baker, Hook and Severinghaus, '44).

Finally, in this connection, what if any function has menstruation as such? It would indeed be comforting to be able to assign it one, but to date no adequate explanation for this excessive breakdown of the uterine endometrium exists. It seems to be merely an overenthusiastic expression in some Primates of the regression following luteal hypertrophy and withdrawal which occurs in a more restrained manner in other more humble Mammals.

Parturition. — This is a process which might naturally be considered at the conclusion of development rather than here. However, possible dependence upon the hormonal substances which we have been discussing makes this an appropriate point to mention the factors which may be involved. As a matter of fact there is not a great deal to say, because comparatively little is really known as to just what factors are actually concerned in this phenomenon. It may be that among others a reduction of progesterone, which quiets uterine contraction, and an increase in oestrogens, which are known to stimulate it, play a part. This,

⁵ Another peculiar effect of these hormones is to cause the release of sperm from the testes of the Frog when so-called pregnancy urine is injected into a lymph sac of one of these animals. This fact furnishes another pregnancy test which promises to be of value (Miller and Wiltberger, '48).

however, is only a guess, and according to Corner many other elements such as the balance of still other hormones, the rate of blood flow through the placenta, the state of nutrition in the fetus, and probably various other conditions are concerned. Indeed some have claimed that the mere size and weight of its tenant finally irritates the uterus into initiating the contractions of labor. Some evidence for this latter notion is perhaps furnished by certain cases in the Cat studied by Markee and Hinsey ('35). In an abnormal situation in this animal one horn of the uterus contained embryos differing considerably in age from those in the other, a condition known as superfetation. In this case the horn with the older fetuses delivered itself thirteen days ahead of the other, the normal full term in this animal being from sixty-three to sixty-five days. This would thus seem to indicate that the conditions responsible for delivery are not entirely hormonal, and hence general, but are at least partly quite local. These investigators also showed that thickness of endometrium and muscle depends on the number and weight of fetuses present in the horn in question. This again emphasizes the effect of local factors on conditions which may affect delivery. In concluding this topic it is pertinent to note the normal term of gestation in the animal we are about to consider in some detail, i.e., the Pig. As usual this period varies slightly with breed and other factors, the range being from 112-115 days, or just under four months (Asdell, '46).

THE SEXUAL CYCLE IN THE MALE

As regards the male among Mammals, it is found that here also there is a tendency toward cycles of sexual activity. This phenomenon, however, is not so common as among the females, or among the males of lower forms. In those species of Mammals in which the male does experience special periods of heightened sexual desire, however, these normally coincide with the breeding season of the female, and are known as the *rutting periods*. At such times the males may develop very special secondary sexual characters, such as the antlers of the buck deer, as well as great irritability and desire for combat with other males. On the other hand, the males of many Mammals have no such special periods of sex activity. Instead, they are apparently able to breed at any time, even though the females of their kind will only receive them at certain seasons.

With this understanding concerning the nature of the sexual cycle and its relation to ovulation and sexual activity, we are now prepared to return to the history of the ovum.

MATURATION AND FERTILIZATION

Although in Mammals the first maturation division often occurs before ovulation and fertilization, the second, with apparently only a few exceptions (e.g., the Mole, Rabbit, and probably Man) occurs after-

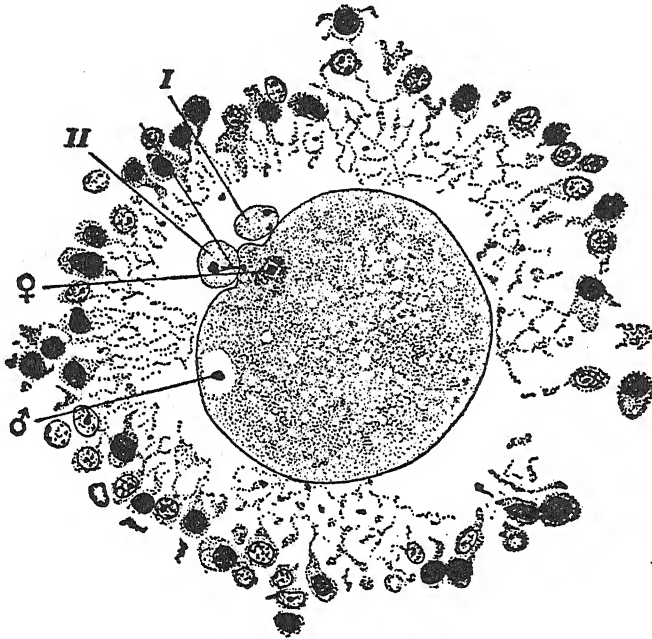


Fig. 250. — Reconstruction of four sections through the fertilized ovum of the Cat. From Longley (combined from two figures). No zona pellucida is visible in these sections. The corona radiata is disintegrating.

s. Remains of second polar spindle. I. First polar body. II. Second polar body. ♂. Sperm nucleus. ♀. Egg nucleus.

ward. Hence it has seemed best to mention both divisions in connection with the latter phenomenon.

The First Maturation Division.—At some time during the growth of the oocyte, the preliminary stages of maturation are completed without any peculiarity of note. The first polar spindle is then formed, and usually a short time before ovulation the first polar body is given off. In the latter connection the only feature to be noted as peculiar to Mammals is the fact that this polar body is normally relatively large, i.e., often as much as one fourth the diameter of the ovum itself,

and in abnormal cases sometimes equal to the latter. The fate of these exceptionally large bodies is not known. After the extrusion of the first polar body, the spindle for the second is formed and moves into position for division. The completion of the process may then take place in the ovary (e.g., in the Mole and Rabbit) or it may be inhibited while ovulation and fertilization occur.

Fertilization. — Sperm introduced into the vagina of the Mammal rapidly make their way into the uterus and up the oviducts. A few hours

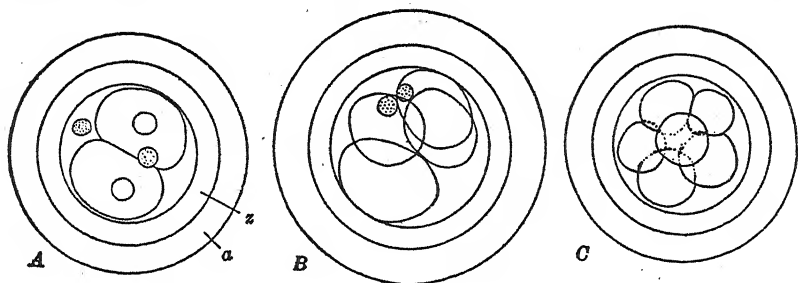


Fig. 251. — Cleavage of the ovum of the Rabbit. From Kellicott (*Chordate Development*). After Assheton. A. Two-cell stage, 24 hours after coitus, showing the two polar bodies separated. B. Four-cell stage, 25½ hours after coitus. C. Eight-cell stage.

a. Albuminous layer derived from the wall of the oviduct. z. Zona radiata.

or even less suffices for them to reach the upper ends of these ducts where the actual process of fertilization usually takes place.

Considerable work has been done on the rate and method of progress of the sperm up the oviducts of different animals. Thus Parker ('31) showed that in the Rabbit the sperm are transported up, both by contractions of the tube and by cilia, despite the fact that the latter beat in an abovarian direction. By contractions the tube is divided into small compartments, and as soon as sperm get into the first of these they are spread throughout it by ciliary currents which move down the walls and up the middle of the compartment. Then the location of the contractions shifts, and new compartments are formed. Sperm do of course swim, but as just suggested, this auto-motility is not the only, or even the main factor, involved in getting them to the upper end of the oviduct. In the Sheep, Schott ('41) found the sperm to reach the upper ends of the ducts in about twenty minutes, and to travel at the rate of 4 cm. (40 mm.) per minute. He does not, however, state that they swim at that rate. Phillips and Andrews ('37) claim an average swimming speed in vitro of only 4.83 mm. per minute over a distance equal to the length of the ewe's

genital tract, though they do much better at first. In the ewe, however, they travel, according to these authors, by swimming or otherwise, at a rate of at least 12.4 mm. per minute. In the Rat, Blandau and Money ('44) say that in twenty-six out of thirty cases sperm reached the infundibulum in forty-five minutes. They do not say just how, but Rossman ('37) suggests a peristaltic activity of the uterus as responsible for movement through that region. In this connection Asdell ('46) also notes that contractions of the uterus probably aid in the transport of the sperm, but gives the "average" time required to reach the infundibulum "in all animals studied" as about four hours. This, it will be noted, is considerably longer than any of the times indicated above, and he does not say what animals were involved. This author further states that none of the first few sperm to reach an egg fertilize it, but they do secrete an enzyme, hyaluronidase, which disperses the cells of the corona radiata, thus making the egg accessible to one of the sperm which follow. He states that about one million sperm at an insemination are necessary to insure fertilization by the one sperm required per egg. This is obviously only a rough estimate, since the kinds of animals, and the numbers of eggs are not given.

Most recently some interesting data have been acquired concerning these matters in relation to Man. These data were presented at the Washington meeting of the American Society of Zoologists ('48) by Dr. E. J. Farris under the title, "Motile Spermatozoa as an Index of Fertility in Man," and the results are quoted with the author's permission. According to this investigator Human sperm swim in vitro at the rate of 3 mm. per minute, a rate not so different for one of those claimed for the Sheep. This author admits, however, that other factors, such as those indicated above, are also active in the movement of the sperm in the female genital tract, and claims that actually they reach the ovum at the upper end in about an hour. This is much better than the "average time in all animals studied" given by Asdell. Farris also notes that at least 130 million motile sperm per c.c. of semen, and preferably more, are necessary to insure fertilization.

Aside from such studies there are others indicating the time which sperm retain their fertilizing capacity. In the Rat, Soderwall and Blandau ('41) say it is at the most fourteen hours, and that it falls off considerably after ten hours. In the Guinea Pig, on the other hand, Soderwall and Young ('40) place the maximum time at twenty-two hours, while in Man, Farris places it at twelve hours, even though the sperm may remain motile much longer than this. An extreme survival time is

found in the Bat where insemination occurs in the fall, and the sperm apparently survive and retain fertilizing capacity in the hibernating females all winter (Wimsatt, '44).

The functional survival of the egg previous to fertilization has also been studied, though not so extensively as in the case of the sperm. It is said, however, to be able to retain its fertilizability for ten hours in the

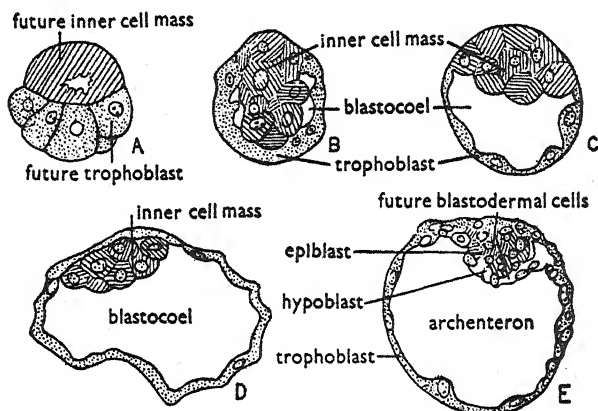


Fig. 252. — Semi-diagrammatic sections through stages of early cleavage, blastula (blastocyst) and early gastrula of the Pig. After Heuser and Streeter. A. Early cleavage. B, C and D formation of blastocyst with inner cell mass. E. Start of epiblast and hypoblast differentiation (gastrulation), probably by delamination, or possibly some infiltration, of cells from the inner cell mass. Trophoblast, often first called subzonal layer.

Rat (Blandau and Jordan, '41), and for twenty hours in the Guinea Pig (Blandau and Young, '39).

From these data it will be evident that even though ovulation may not occur so that an egg is present at the moment sperm reach the upper end of the oviduct there is still good opportunity for fertilization to occur there over a reasonable period. When a viable sperm does reach an egg it makes its way through any remaining cells of the corona radiata and through the zona pellucida which still cover it. Usually only one actually enters the egg, presumably due to mechanisms similar to those previously described. In many cases, only the head and middle piece of the sperm enter, but in others (Mouse), the entire spermatozoon is taken in; when this does occur, however, the tail soon degenerates. The head of the sperm next forms the sperm nucleus (male pronucleus) in the usual manner.

The Second Maturation Division. — If this has not already been completed its completion occurs following the entrance of the sperm and while the nucleus of the latter is forming; it results in a second polar body, usually smaller than the first. This division is soon followed by the union of the sperm and egg nuclei, and the process of fertilization is complete (Fig. 250).

SEGMENTATION, GASTRULATION, AMNION FORMATION, AND THE PRIMITIVE STREAK

SEGMENTATION

The Type of Cleavage. — Segmentation in the placental Mammals is total, as might be expected from the virtual absence of yolk. The arrangement and behavior of the cells, however, is quite different from that observed in the first yolkless form which was studied, i.e., *Amphioxus*. The reason for this is apparently due to the fact that the egg of a Mammal is almost certainly only secondarily without yolk. The evidence for this assumption will become more and more obvious in the course of this chapter, but a couple of the more striking proofs may be indicated here. Thus as will appear, the embryos of the primitive non-placental Mammals known as *Monotremes* possess both yolk-sac and yolk, while all the placental Mammals retain the sac, though it is empty. Secondly, there are the origin of the embryo from what amounts to a blastoderm, the method of gastrulation, and other features all characteristics of large-yolked forms. We may now proceed to the actual method of segmentation.

The Blastocyst. — Cleavage, though total, is irregular from the start (Fig. 251). The result is the formation of a spherical mass of cells known as the *morula* in which the cells are of two types. On the outside they are at first cubical, but soon assume the form of a flattened epithelium, which being covered temporarily by the *zona radiata* is called the *subzonal layer*, later the *trophoblast*. The cells on the inside, on the other hand, are spherical and are called the *inner cell mass*. Presently, vacuoles appear on one side of this mass, beneath it and the subzonal layer. These run together and increase until more than half of the morula is occupied by a fluid-filled cavity. On the other side, the inner mass hangs from the wall like a suspended drop (Fig. 252). The morula has now become a *blastodermic vesicle* or *blastocyst*, which corresponds in a general way to the blastula of lower forms. Hence the cavity may

be termed the *blastocoel* or *subgerminal cavity*, while the fluid within it occupies the place of the yolk. Finally, as subsequent development shows, the inner cell mass lying above the fluid virtually plays the part of a *blastoderm* (Fig. 253).

Cleavage occurs while the ovum is passing down the oviduct, and in some instances it may even have reached the blastocyst condition by the time it arrives in the uterus. The time required for this passage varies

much in different animals, but is ordinarily considerable, e.g., about four days in the Rabbit, and eight or ten days in the Dog. The movement down the duct is apparently accomplished mainly by peristaltic action, though in the Rabbit, Parker claims that the cilia beating in an abovarian direction are involved.

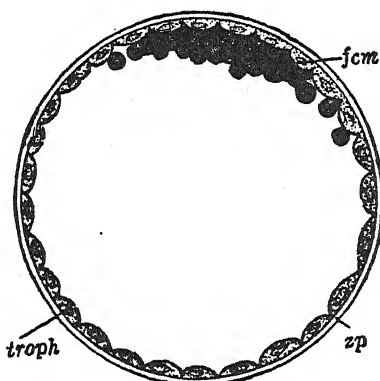


Fig. 253. — Section through the fully formed blastodermic vesicle of the Rabbit. From Quain's *Anatomy*, after Van Beneden.

f.c.m. Granular cells of the inner cell mass. troph. Trophoblast. zp. Zona pellucida.

Within the uterus the cleaving egg, or morula, soon becomes a blastocyst, if it is not already one, and this begins to enlarge through the multiplication and flattening of the cells of the subzonal layer (Fig. 253). There is considerable variation in the size and shape which is reached in this manner.

Thus in the Rabbit, the vesicle after three days in the uterus becomes ovoidal, measuring about 4.5 x 3.5 mm. In Ungulates, on the other hand, it becomes very long and tapering, that of a nine day Pig measuring about 8 cm. in length and .5 mm. in diameter, while in a day or two more the length has reached about a meter, and the diameter a few millimeters. In all cases, however, the inner cell mass remains very small, and in instances where the vesicle is elongated, as in the Pig or Sheep, the mass is attached about midway between its ends (Fig. 254).

GASTRULATION

As in the other forms studied, this term is here used to denote the formation of an *archenteric cavity*, and the setting aside of *epiblast* and *hypoblast*. In most Mammals the latter appears to arise either by a splitting off (delamination) of cells from the ventral side of the inner cell



Fig. 254.—Photographs of Pig blastocyst by Heuser and Streeter showing the transition from an oval to an elongated form. In group *A* the long axis of the smallest specimen was approximately 7.5 mm., while in the largest it was about 13.8 mm. In group *B* the magnification is less so that the smallest specimen on the extreme left actually measured about 15 mm. in length, and the greatly elongated specimen at the top of the group measured about 150 mm.

mass, or by an infiltration of cells from this area. It will be recalled that both these possibilities are identical with some of those recently suggested as occurring in the origin of the primordial hypoblast of the Chick. At all events the cells so produced then multiply and spread around the inside of the vesicle until in many forms they eventually completely line it, just as they line the archenteron and yolk-sac of the Bird. This extension of the hypoblast and later mesoderm around the inside of the blastocyst is of course essentially epibolic, though the overgrowth covers only a cavity. The cavity so lined constitutes the *archenteron*, while part of it presently becomes the *yolk-sac* in a man-

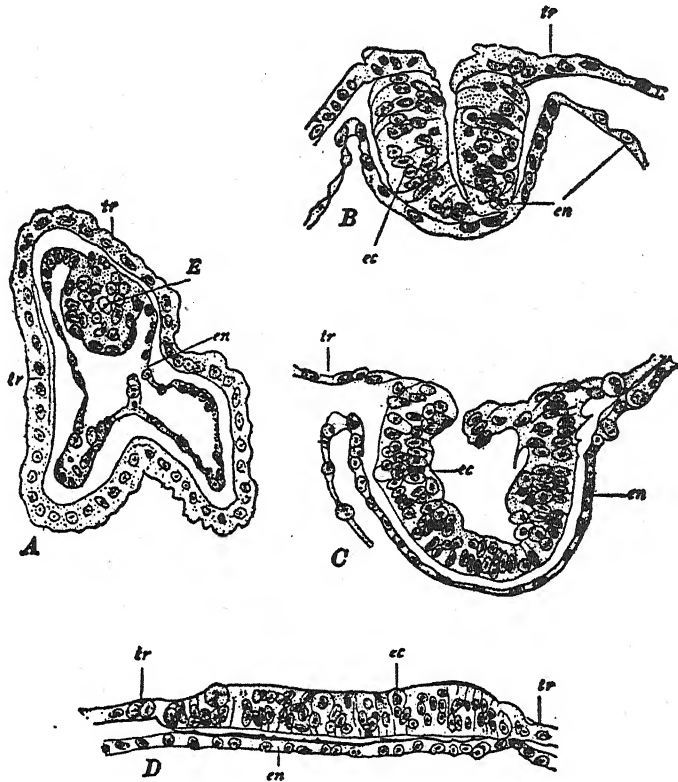


Fig. 255. — Sections through four stages in the early development of the Insectivore *Tupaia javanica*. From Hubrecht. A. Blastodermic vesicle completely closed; hypoblast still continuous with the embryonic epiblast. B, C. Embryonic epiblast split and folding out upon the surface of the vesicle, pushing away the trophoblast cells. D. Embryonic epiblast forming a flat disc on the surface of the blastodermic vesicle.

E. Inner cells mass, now embryonic knob. ec. Embryonic epiblast. en. Hypoblast. tr. Trophoblast.

ner to be indicated, despite the absence of yolk. Thus the situation differs from that found in previous forms, and particularly in the Bird, as follows: In the latter case the original archenteron consisted only of a shallow space between the hypoblastic roof and the underlying yolk. The central region of the roof, later augmented by mesoderm, then folded off to form the gut, while the borders grew out and around the yolk to form the sac. In most Mammals, on the other hand, there is of

course no yolk at all, so that the cavity of the blastocoel beneath the hypoblast may all, at first, be called archenteron. Later on the hypoblastic roof of this cavity now accompanied by mesoderm, and hence termed *endoderm*, folds off as in the Bird to form a gut. Meanwhile the remainder of the cavity may or may not have become completely lined with endoderm. In the Guinea Pig for example only the roof is ever so constituted. In any event the part of this cavity not eventually occupied by the allantois, amnion and extra-embryonic coelom becomes the *yolk-sac*, with or without a ventral wall. In many cases, as in the Rabbit, Cat and Pig, this sac is fairly extensive, especially at first. In others, like most Primates, it is very insignificant. Certain special details and peculiarities of these extra-embryonic structures will be considered later. Meanwhile it is to be noted that with the origin of the hypoblast the remainder of the inner cell mass together with the original subzonal layer may now be termed the epiblast. This epiblast is then further divided into that which composes the inner cell mass proper, now termed the *embryonic knob*, and that which composes the subzonal layer, now termed the *trophoblast*. It is to be noted that the latter completely encloses, for a time at least, the embryonic knob and the yolk-sac. Hence though originating differently, it occupies the same position as the chorionic ectoderm of the Chick (Fig. 255, A). In fact, with the mesoderm which in some cases later comes to line it, this layer constitutes the *chorion* of the Mammal.

It is to be clearly understood that the process of gastrulation which has just been described is entirely one of delamination or infiltration, and proliferation; there is apparently no involution, invagination, nor epiboly, and hence also no concrescence. Consequently, it is not surprising that there is no well marked *blastopore*, at least in connection with the actual process of hypoblast formation. Later, as in the Chick, a primitive streak arises as a thickening in the epiblast, and again as in the Bird, parts of this streak are interpreted by many as the homologue of a blastopore. This will be discussed further when the origin of the primitive streak is described.

IMPLANTATION

By the time the stage described above has been reached, and sometimes somewhat earlier, the blastocyst has become attached to the uterine wall. This process is known as *implantation*, and there are several methods by which it is brought about. It will be best, however, to postpone their detailed discussion until the description of the placenta is

taken up. Suffice it to say at this point that it is brought about largely by the activity of the trophoblast, aided by certain changes in the uterine wall itself.

THE AMNION

There are two chief methods by which the amnion is formed in the Mammal:

I. The First Method of Amnion Formation.—This method may be defined briefly as the method of amnion formation by folds. The

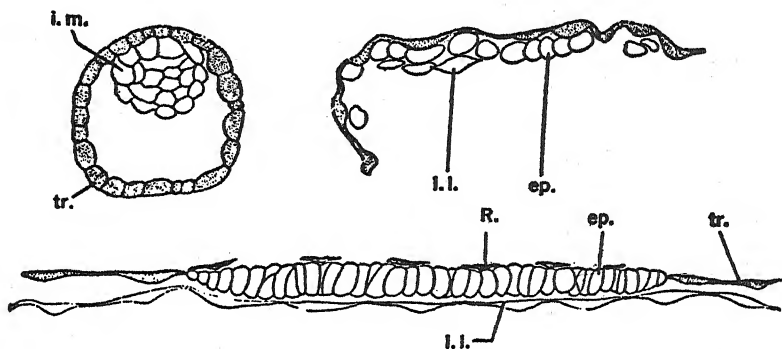


Fig. 256.—Formation of the amnion in the Rabbit (*Lepus*). From Jenkinson (*Vertebrate Embryology*). After Assheton.

i.m. Inner cell mass. *i.l.* Lower layer (i.e., hypoblast). *ep.* Embryonic plate (i.e., blastodermal epiblast). *R.* Cells of Rauber. *tr.* Trophoblast.

first step in this method involves the transformation of the epiblast of the embryonic knob into a flattened plate overlying the hypoblast, the two layers being virtually homologous with the similar ones of the avian blastoderm. This flattening is accomplished, however, by two different processes. Thus though subsequent development of the amnion itself is similar, it is convenient upon the basis of the above differences in the initial stages to describe Method I under two headings, Type (a) and Type (b).

Method I, Type (a).—This type is illustrated by one of the Insectivores, *Tupaia* (Fig. 255); in this animal a depression appears in the top of the embryonic knob, and extends well down into it. The bottom of the depression then rises to the surface, and the edges are at the same time pushed apart. As this occurs the trophoblast cells above are broken and scattered. Thus the epiblastic plate of the blastoderm so formed comes to lie directly on the surface of the blastocyst.



Fig. 257. — Differentiation of the early Pig blastoderm. After Heuser and Streeter. *A*, *B* and *C* are from blastocysts measuring .6 mm. in diameter, and show clear differentiation of the inner cell mass (chiefly epiblast), and a thin layer of hypoblast, the whole being covered by a layer of trophoblast. *D* measured .8 mm., but does not show the hypoblast. The trophoblast over the inner cell mass is scattered, only two cells (cells of Rauber) remaining.

Method I, Type (b). — In this type, of which the Rabbit or the Pig form equally good examples (Figs. 256, 257), the process is simpler, for here the knob merely flattens without the occurrence of any previous depression. In such cases after the flattening is completed, scattered trophoblast cells may remain for a time over the blastoderm, and are known as the cells of Rauber; these, however, soon disappear.

Subsequent Stages of Method I, Types (a) and (b). — As suggested above it will now appear that the later stages of types (*a*) and (*b*) are virtually alike. Before they are described, however, it should be noted that during or soon after the above processes, mesoderm has been proliferated between the epiblast and the underlying hypoblast in a man-

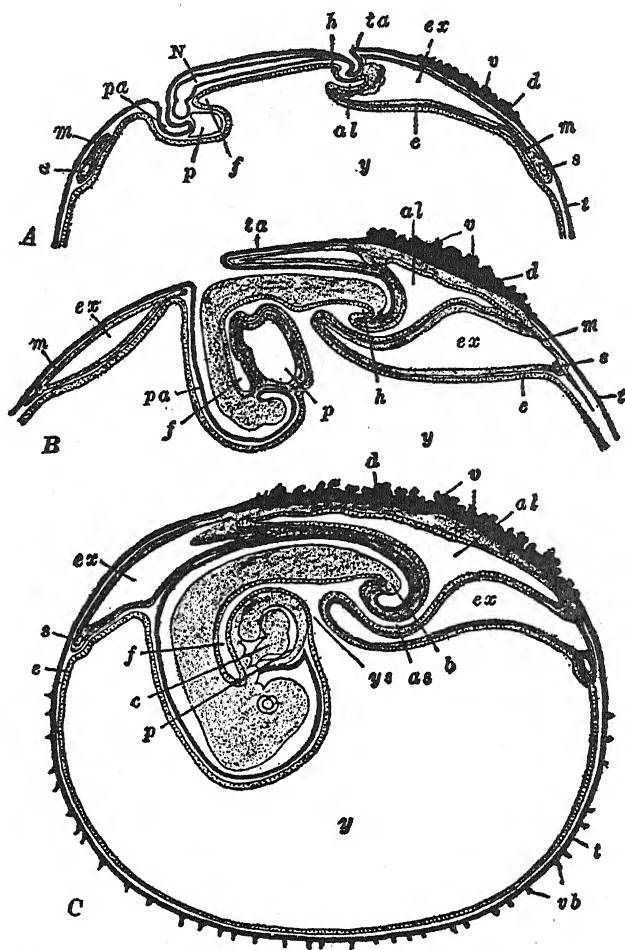


Fig. 258. — Diagrams of the formation of the embryonic membranes and appendages in the Rabbit. From Kellicott (*Chordate Development*). After Van Beneden and Julin (partly after Marshall). Sagittal sections. A. At the end of the ninth day, after coitus. B. Early the tenth day. C. At the end of the tenth day. Ectoderm black; endoderm dotted; mesoderm gray.

al. Allantois. as. Allantoic stalk. b. Tail-bud. c. Heart. d. Allantoidean trophoderm (see page 543). e. Endoderm. ex. Exocoelom. f. Fore-gut. h. Hind-gut. m. Mesoderm. N. Central nervous system. p. Pericardial cavity. pa. Proamnion. s. Marginal sinus (sinus terminalis). t. Trophoblast. ta. Tail fold of amnion. v. Trophodermal villi. vb. Trophoblastic villi. y. Cavity of yolk-sac. ys. Yolk-stalk.

ner to be described below. The two first layers may henceforth therefore be referred to as *ectoderm* and *endoderm*. Moreover, there has arisen within this mesoderm the usual coelomic split, separating it into the somatic and splanchnic layers. In either type (a) or (b), the amnion is then formed by folds of ectoderm and somatic mesoderm, which arise about the rim of the flattened embryonic knob (i.e., the blastodermal ectoderm), in essentially the same manner as in the Chick (Fig. 258). Thus as the amnion is completed by the meeting of the folds at the sero-amniotic connection, the chorion is at the same time re-established above it. This portion of re-established chorion now consists as usual therefore not only of an outer layer of ectoderm, but also of an inner layer of somatic mesoderm. Between the latter and the somatic mesoderm of the amnion is of course the extra-embryonic coelom.

There are, however, certain minor points of difference to be noted between the case of the Bird and that of the placental Mammal. In the first place there is the origin of the chorionic ectoderm. In the Bird this arises entirely from ectoderm of the extra-embryonic blastoderm which has grown out over the yolk. In the Mammal, on the other hand, since the folds arise just at the border between blastodermal ectoderm (embryonic knob) and trophoblast, a large portion of the ectoderm in the folds, i.e., that of the outer layer, seems to be formed from the latter substance. Thus while the lining of the amnion may be chiefly blastodermal, the ectodermal part of the chorion which covers it is apparently entirely of trophoblast, a tissue which seems to have no real homologue in the Bird. A second but rather less important difference between Bird and Mammal is the fact that in the latter the tail fold often appears earlier than the head fold, and is therefore the longer of the two. In the Pig, on the other hand, head and tail folds are virtually equal, and are continuous with the lateral folds which arise coincidentally (Fig. 300).

II. The Second Method of Amnion Formation.—In the second method of amnion formation, the trophoblast above the embryonic knob is never interrupted, a condition known as *entypy*. In contrast to Method I, the amniotic cavity then arises merely as a space within the embryonic knob or in connection with the knob and the trophoblast above it. Here again, however, there are variations in the process, so that it may best be described under the headings, Type (a), Type (b), and Type (c).

Method II, Type (a).—This type is illustrated by the Hedgehog (*Erinaceus*, Fig. 259) in which the rudimentary amniotic cavity appears, not in the knob itself, but as a space between the center of its dorsal side

and the trophoblast. The edges of the knob, however, remain adherent to the trophoblast, and these edges now turn and grow toward one another between the trophoblast and the cavity. Thus when they meet and fuse, the epiblastic (future ectodermal) layer of the amnion is completed. Later, the extra-embryonic coelom lined by mesoderm forces its way in between the trophoblast (now chorionic ectoderm) and the epiblast, now ectoderm, of the amnion, so that in this manner the latter receives its mesodermal covering and the former its mesodermal lining. It

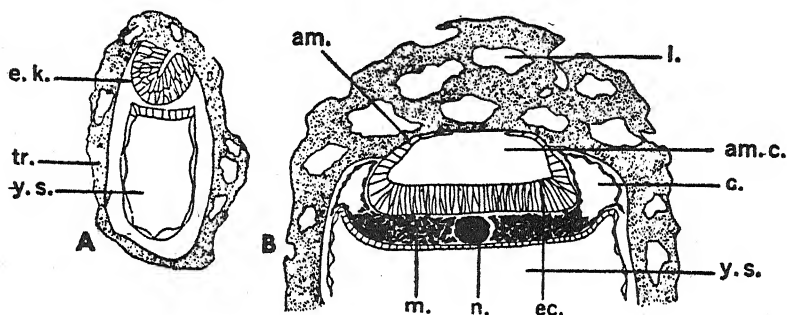


Fig. 259. — Formation of the amnion in the Hedgehog (*Erinaceus*). From Jenkinson (*Vertebrate Embryology*). After Hubrecht. A. Early. B. Later stage.

am. Amnion. c. Extra-embryonic coelom. ec. Ectoderm. e.k. Embryonic knob. l. Lacuna. m. Mesoderm. n. Notochord. tr. Trophoblast. y.s. Yolk-sac.

may be noted that the type of amnion formation thus exemplified by the Hedgehog is quite similar in many respects to that just described under Method I, and may, therefore, represent a transitional stage between Methods I and II. Later, as the embryo develops, the edges of the flat blastoderm are folded downward in the usual manner, and portions of the mesodermal layers are of course involved in this process. The layer lying next to the endoderm is then splanchnic mesoderm, and the one next to the ectoderm (either trophoblastic or embryonic) is somatic mesoderm.

Method II, Type (b). — The second type of Method II is typically illustrated in the development of the Guinea Pig (*Cavia*), in which the process is as follows:

Shortly after gastrulation is completed, the embryonic knob becomes separated from the trophoblast above it, and moves down near the opposite side of the blastocyst.⁶ In so doing, it pushes the central portion

⁶ In this case and that of the Mouse and Rat the blastocyst, presumably because of its shape, has been termed by some the "egg cylinder," though it is of course neither an egg nor a cylinder.

of the hypoblast layer before it; the edges of this central portion, nevertheless, remain attached to the dorsal trophoblast. This process presently results in the production of a clear space between the knob and the trophoblast, bounded on its sides by the upstretching hypoblast. A cavity now develops in the middle of the embryonic knob; this is the rudiment of the amniotic cavity (Fig. 260, *A, B*). On the floor of this cavity, the cells remain columnar, and are homologous with the upper

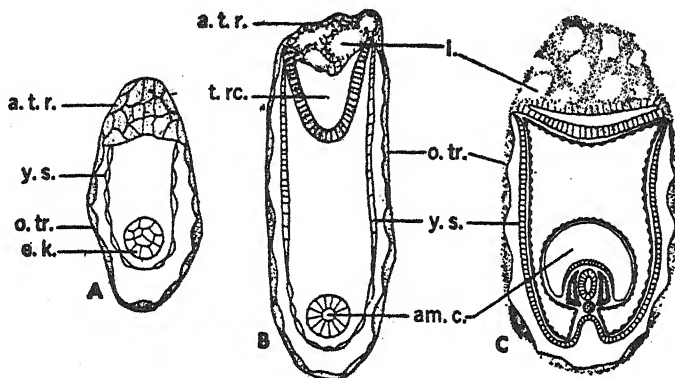


Fig. 260.—Formation of the amnion in the Guinea Pig (*Cavia*). From Jenkinson (*Vertebrate Embryology*). After Selenka. *A*. Early. *B*. Later. *C*. Latest stage.

a.tr. Allantoidean trophoblast. *o.tr.* Omphaloidean trophoblast (see page 543). *l.* Lacuna. *e.k.* Embryonic knob. *am.c.* Amniotic cavity. *y.s.* Yolk-sac hypoblast in *A* and *B*, endoderm in *C*.

or epiblastic layer of the embryonic portion of the blastoderm in previous forms. The cells of the roof and sides, on the other hand, soon flatten and form the epiblastic layer of the amnion. The latter now begins to expand, filling the space above it (Fig. 260, *C*). In the meantime mesoderm begins to arise between the epiblast of the blastoderm and the hypoblast beneath it. Thus the former becomes ectoderm and the latter endoderm, while within the mesoderm the coelomic split occurs, producing two layers. These layers then spread out upon either side, the lower layer extending over the endoderm as the splanchnic mesoderm, and the upper layer extending up over the ectoderm of the amnion as the somatic mesoderm. The amnion is now completely formed, and consists, as in previous cases, of an outer layer of mesoderm and an inner one of ectoderm. Further development merely involves an increase in size and a gradual folding in about the embryo to form the umbilical stalk.

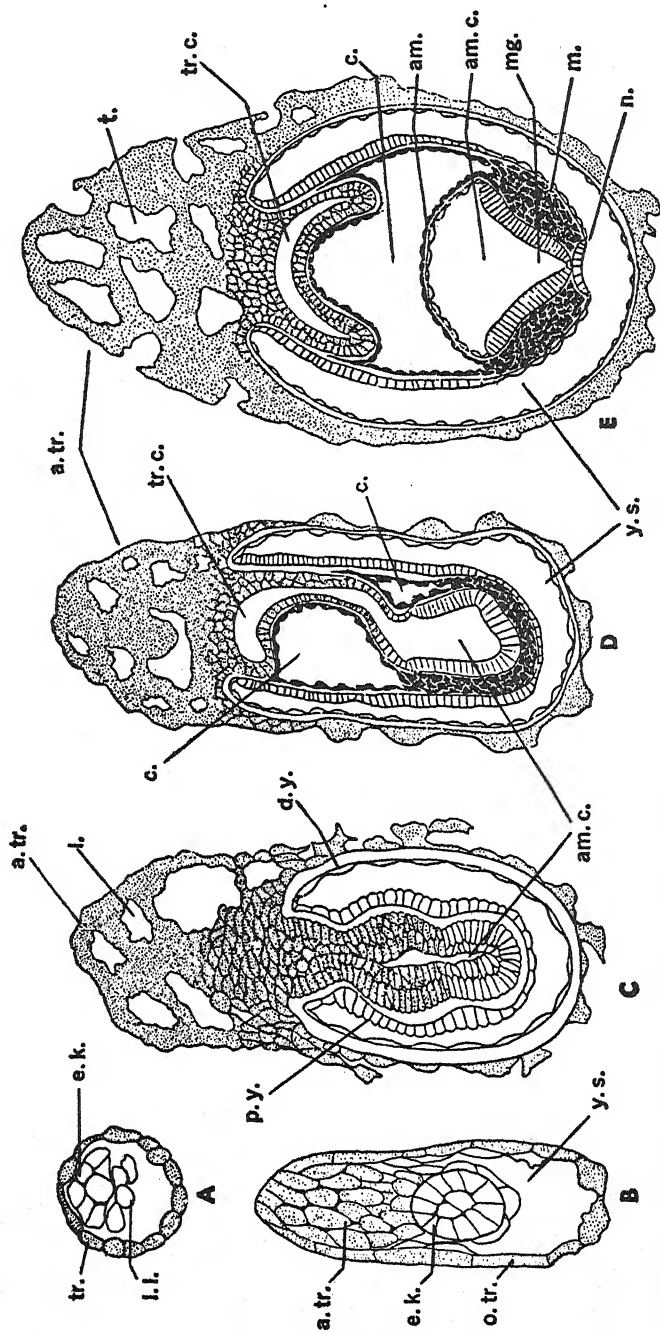


Fig. 261. — Formation of the amnion in the Mouse (*Mus*). From Jenkinson. (*Vertebrate Embryology*). A-E. Successive stages. *am.* Amnion. *am.c.* Amniotic cavity. *a.tr.* Allantois. *e.c.* Extra-embryonic coelom. *e.k.* Embryonic knob. *l.* Lacuna. *l.l.* Lower layer, i.e. hypoblast. *m.* Mesoderm. *m.g.* Medullary groove. *n.* Notochord. *o.tr.* Omphaloidean trophoblast. *py.* *dy.* Proximal or upper, and distal or lower walls of yolk-sac. *tr.* Trophoblast. *tr.c.* Temporary trophoblastic or false amniotic cavity. *y.s.* Yolk-sac.

In anticipation of the method which is next to be described under type (c), however, it may finally be added that besides the amniotic cavity thus formed, there has also arisen a cavity in the dorsal trophoblast from which the knob was separated. This second space is often referred to as the *false amniotic cavity*, but in the type under discussion it never has any connection with the true cavity. It presently disappears and has no further significance.

Method II Type (c). — This last type of amnion formation is well shown in the Mouse (*Mus*, Fig. 261). In this form the embryonic knob moves down as in the Guinea Pig, pushing the endoderm before it, but does not become separated from the trophoblast. Instead, the latter simply thickens, thus filling up the space which would otherwise result. A cavity now appears in the upper part of the knob, and at once comes into communication with a cavity in the lower part of the thickened trophoblast, i.e., the false amniotic cavity. The mesoderm next arises between the hypoblast, now endoderm, and the epiblast, now ectoderm, of the knob, whence it spreads upward between the endoderm and the thickened trophoblast. Within this mesoderm the coelomic split next develops upon either side, and the two coelomic spaces then press toward each other and finally unite. In this manner the mass of ectoderm and trophoblast, including the cavity, is cut in two in approximately the region where the ectodermal and trophoblastic elements were in contact. This process is such as to leave one closed cavity in the trophoblast and another closed cavity in the embryonic knob, with the extra-embryonic coelom lined by mesoderm between them. The cavity in the knob is, of course, the amniotic cavity with its usual layers, while the one in the trophoblast is the false cavity already referred to. The latter, it will be noted, is in no wise different from its homologue in type (b), except that in this case it temporarily communicates with the true cavity. Later, as in the former case, it disappears.

The Inversion of the Germ Layers. — Before passing on to a discussion of the relative primitiveness of Methods I and II, it is worth while to note a peculiar misconception which arose in the minds of early students of forms like *Cavia* and *Mus*. These are cases, it will be recalled, where the embryonic knob moves far down into the blastocyst. The obvious result is that the endoderm extends well up on either side, considerably above the level of the blastoderm. Hence, if in examining the blastocyst of such a form, the investigator overlooked the outer layer of trophoblast, the first layer he would come to would be endoderm. He would thus get the impression that in some mysterious manner the endo-

derm had gotten on the outside of the blastocyst. This oversight was exactly what occurred, and the phenomenon was, therefore, referred to as an "inversion of the germ layers." As a matter of fact, it is now clear that no such inversion really exists, and hence the phrase is of only historical interest.

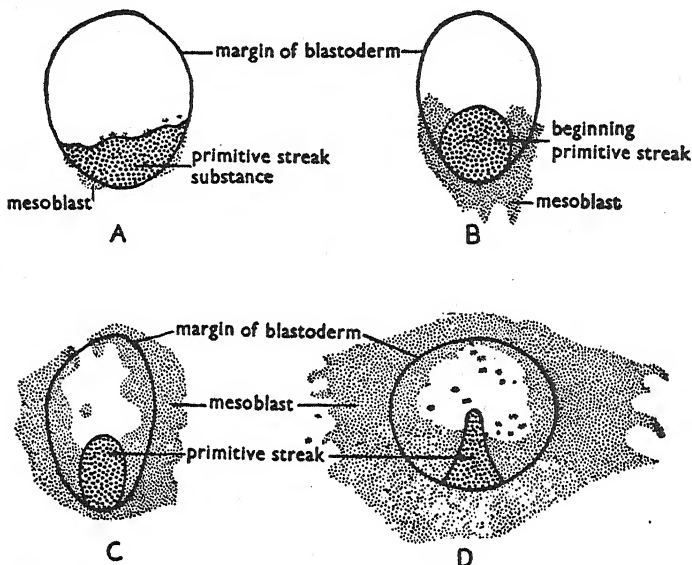


Fig. 262. — Graphic reconstructions of the Pig blastoderm in the pre-streak and early streak stages. After Streeter. *A.* Pre-streak stage. *B.* Early primitive streak, showing beginning mesoblast formation. *C* and *D.* Later stages in primitive streak development with greater extension of the mesoblast. As in the Chick, the mesoblast can be seen spreading out from the sides of the streak.

The Relative Primitiveness of Methods I and II. — There has been some discussion as to which of these two main methods of amnion formation is the more primitive among placental Mammals, one view — that of Hubrecht — being strongly in favor of Method II. The reasons for this attitude are based chiefly upon the characteristics of the mammalian chorion indicated in connection with Method I, and are as follows: In the Bird or Reptile (i.e., the Sauropsids), there is, as suggested, no chorion (the layer corresponding in relative position to the mammalian trophoblast) until it is formed by the outer walls of the amniotic folds. In all the Mammals whose early development is known, on the other hand, the blastocyst is entirely enclosed in trophoblast, or chorionic epiblast, before any amnion has been formed, either by folds or

otherwise. It is true that in those cases where the process of folding occurs (e.g., in the Rabbit), the original trophoblastic chorion above the embryo virtually disappears, and the new one in this region is formed from the outer walls of the folds. Nevertheless, even in these cases there is no denying that there was a trophoblastic chorion previous to the

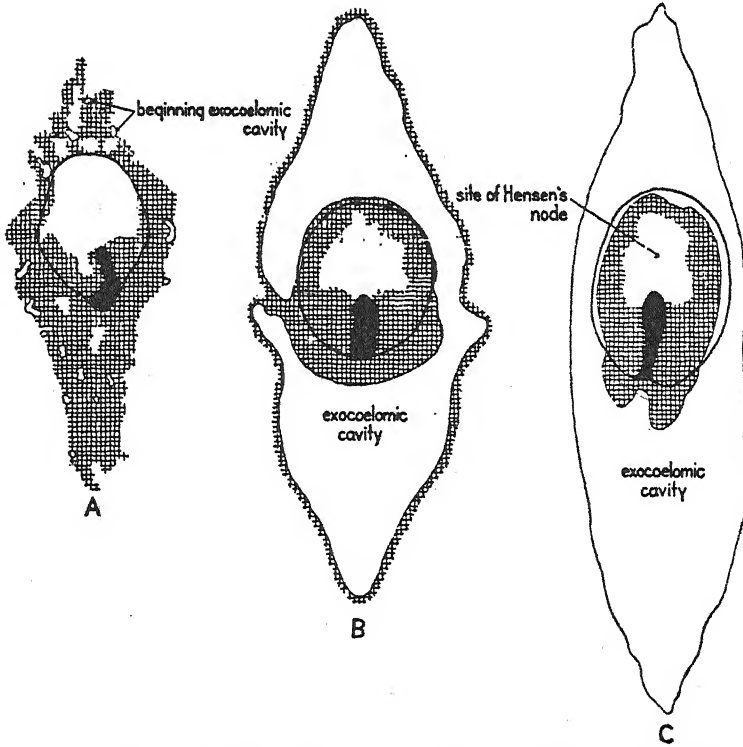


Fig. 263. — Later primitive streak and mesoblast formation in the Pig. After Streeter.

folding, and further that most of the (chorionic) portion of the folds is still really trophoblastic. Hence, as indicated above, it is said that the original trophoblastic chorion of Mammals cannot be regarded as homologous with the layer of the same name in the Sauropsids. From this statement it then follows, according to proponents of this idea, that the cases of the formation of the mammalian amnion and chorion by folds could not have been derived from this process in the Reptiles; it must rather represent a reversion to the reptilian condition, or else a piece of independent evolution.

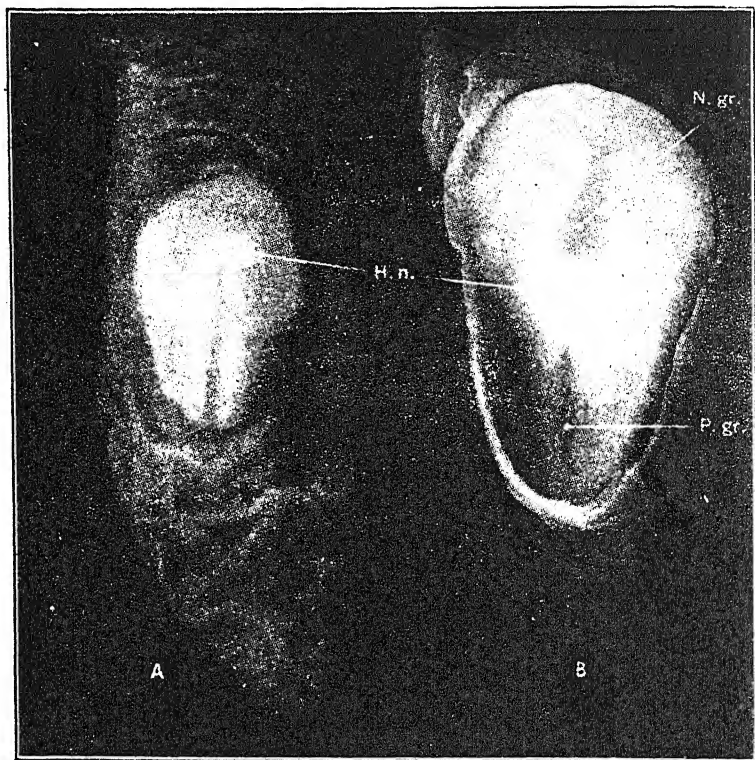


Fig. 264. — Surface view of two stages of the Pig blastoderm with parts of the adjacent blastocyst. After Streeter. *A*. Primitive groove stage, length of blastoderm about 1 mm. *B*. Blastoderm showing primitive groove and also beginning neural groove, length 1.7 mm. Crest of chorio-amniotic fold shows around margin of blastoderm.

H.n. Hensen's node (knot). *N.gr.* Neural groove. *P.gr.* Primitive groove.

There are, however, many zoologists who do not subscribe to the theory just presented. Instead they regard Method I as the more primitive, for the following reasons: In the first place it is known that Mammals as a class sprang from Reptiles, in which group the method of amnion formation is by folds as in the Birds. Furthermore, among those Mammals which are in other respects most primitive, i.e., the Monotremes and Marsupials, the formation of the amnion by folds (according to the evidence of those stages which are known in these animals) in all probability prevails. Lastly, as admitted by the opponents of the view now being presented, the trophoblastic chorion of the Mammal is not really homologous with the true chorion of the Bird; it is rather a secondary develop-

ment, whose early and complete enclosure of the blastocyst is made possible by the absence of yolk. Consequently, though the trophoblast usually takes a large part in the formation of the mammalian chorion, it has not, contrary to the argument stated in the foregoing paragraph, necessarily anything to do with the formation of the amnion. Indeed, as has been seen, the latter frequently forms by folds in spite of the presence of the precocious trophoblastic chorion, and those cases where it does not (Method II) are merely another secondary development. In conclusion, it may be said that on the whole the arguments for the conception just presented appear to be rather more cogent and reasonable than those opposed to it and it is the one which is more widely held.

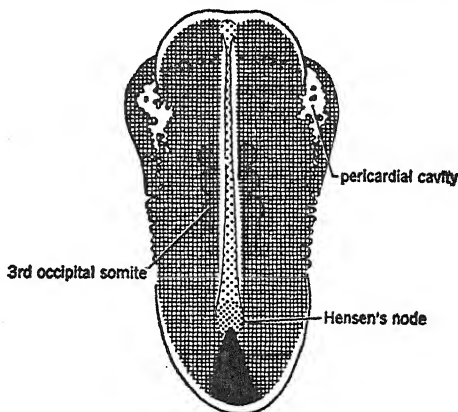


Fig. 265. — Reconstruction of a surface view of a Pig blastoderm, length 1.56 mm. After Streeter. Heavy dotted line anterior to Hensen's node is the notochord. Cross hatched region is mesoderm. Darkly lined area posterior to Hensen's node is remains of primitive streak.

THE PRIMITIVE STREAK AND RELATED STRUCTURES

It will have been noted that during the process of amnion formation (in Method I, slightly preceding it) there arises in one way or another from the embryonic knob a flat plate of epiblast. This area of epiblast together with the hypoblast directly beneath it is the area from which the embryo proper is now to develop. As has been suggested, in the Chick it is termed the embryonic blastoderm; in the Mammal it is the *embryonic disc*.

The Primitive Streak and Groove. — The *primitive streak* arises along the mid-line of the embryonic disc in what later proves to be the longitudinal axis of the embryo. The questions as to its source are very much the same as they were in the case of the Chick, but not so much experimental work has been done in an effort to answer them. The reasons for this are fairly obvious in view of the conditions under which the Mammalian embryo develops. However, careful study of fixed material has been made by Streeter and others in the case of the Pig, and

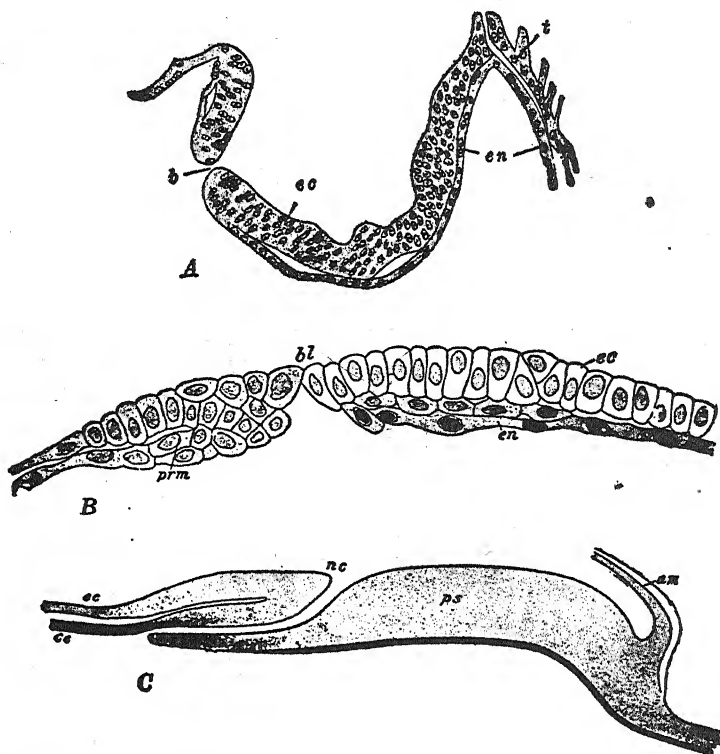


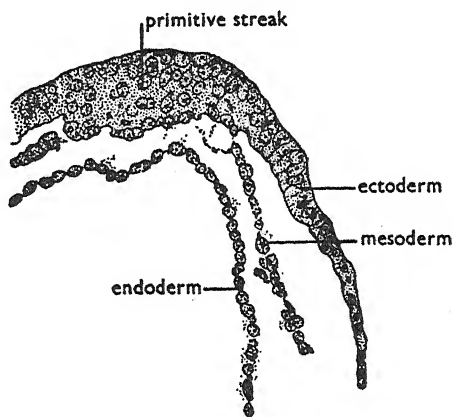
Fig. 266. — *A*. Sagittal section through the embryonic shield of the Hedgehog, showing the transitory blastopore. From Kellicott (*Chordate Development*). After Hubrecht. *B*. Posterior part of a sagittal section through the embryonic disc of the Mole. *C*. Diagram of a sagittal section through the embryonic disc of the Mole. From McMurrich (*Development of the Human Body*). After Heape.
am. Amnion. *b* or *bl*. Blastopore. *ce*. Chorda endoderm. *ec*. Ectoderm. *en*. Endoderm. *nc*. Neurenteric canal. *prm*. Peristomial mesoderm. *ps*. Primitive streak. *t*. Trophoderm.

the following conclusions seem justified. There first appears a thickened crescent of epiblast about what proves to be the posterior margin of the disc (Fig. 262, *A*). This crescent then assumes the form of an oval (Fig. 262, *B*, *C*), and this gradually elongates into the *primitive streak* (Fig. 262, *D*; Fig. 263). Presently, as in the Bird, a *primitive groove* forms along the middle of the streak and at its anterior end there develops a thickened spot, *Hensen's knot* (Figs. 264, 265). It is to be particularly noted that in this knot there is likewise a *pit* which in some Mammals, e.g., the Hedgehog, as in some Birds, temporarily opens into the archenteron (Fig. 266). In some others the pit merely pushes into

the notochord where it is known as the *notochordal canal*. In either case its possible homology with the part of the blastopore which in other cases forms a neurenteric canal is obvious, even though it disappears before the neural folds arise. Just what is going on during these changes of shape from a crescent, to a streak with a groove and knot, is not certain. It seems highly probable, however, that the process is again one of convergence of material toward the mid-line, and perhaps even some concrescence. Also as in the Chick, there is apparently rapid proliferation of cells in this region. The meanings of the groove and knot are no more or less clear than in the case of the Chick, and whatever their significance in that form they probably have the same significance in the Mammal (see below).

Origin of Mesoderm and Notochord. — As in the Chick, so in the Pig, and presumably in other Mammals, the streak is again the source of the *mesoderm*, which is proliferated from its sides, and spreads out on either hand and posteriorly

(Figs. 267, 268). Indeed as shown in Figure 262, this proliferation actually begins even before the streak primordium has assumed its definitive elongated form. Whether there is later any actual movement of cells through the streak from the upper surface, i.e., anything like infiltration (involution), as was suggested in the case of the Bird is not known, but it seems quite possible. If this were true it might help, again as in the Bird, to account for the development of the groove. Be that as it may the mesoderm having thus originated as a single sheet, very early begins to split into the usual somatic and splanchnic layers. This splitting starts in random isolated areas, thus producing small vesicles, which presently coalesce, to form more extensive coelomic spaces (Figs. 262, 263). It will be noted incidentally that the coelom first formed in this manner actually lies outside the definitely embryonic area, i.e., ap-



x section pig blastoderm

Fig. 267. — Transverse section of one side of a Pig blastoderm similar to one from which surface reconstruction in Fig. 262, C, was made. After Streeter. Long axis measurement of the blastoderm from which this section was taken was .5 mm.

proximately the region comparable to the area pellucida of the Chick. Hence this first coelomic space is extra-embryonic, but very shortly it spreads within the embryonic region. Finally the *notochord* (head-process) of the Pig arises according to Streeter ('27) as a rod of cells

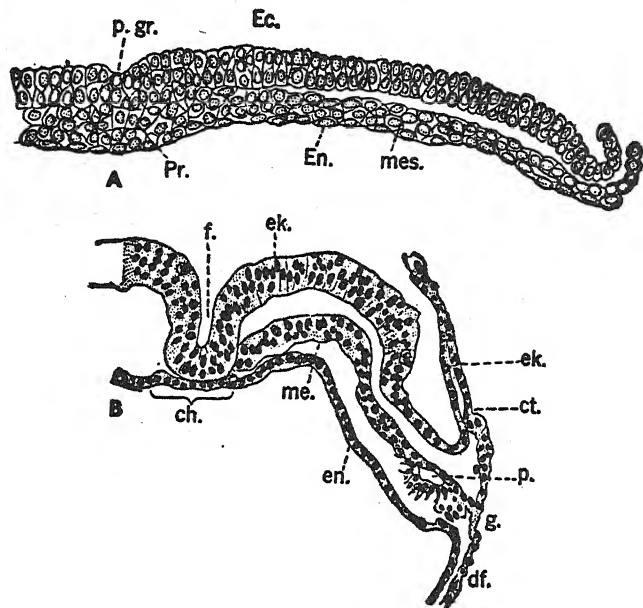


Fig. 268. — *A.* Transverse section through the primitive streak of the Mole. *B.* Transverse section through a Human embryo of 1.54 mm. (Graf von Spee's Embryo Gle.) From Minot (*Laboratory Text-Book of Embryology*), after Heape (*A.*), and Graf von Spee (*B.*).

ch. Notochord. *ct.* Somatic mesoderm of amnion. *df.* Splanchnic mesoderm. *Ec.* or *ek.* Ectoderm. *en.* or *En.* Endoderm. *df.* Dorsal furrow. *g.* Junction of extra-embryonic somatic and splanchnic mesoderm. *me.* or *mes.* Mesoderm. *p.* Rudiment of embryonic coelom. *p.gr.* Primitive groove. *Pr.* Primitive streak.

proliferated at the primitive knot and pushed anteriorly. This it will be recalled is identical with one of the theories of notochord origin in the Chick. According to one of the most recent theories, however (Spratt, '47), the notochord in the Bird lengthens by growing posteriorly rather than anteriorly, as the primitive streak shortens. It is quite probable that whatever the true process proves to be in that case it will be found to hold also for the Mammal. However that may be, it should be noted that there is an interesting difference between the relation of the mesoderm and notochord in the Pig from that observed in the Chick. Thus it

will be seen that in the Pig the notochord has no mesoderm free area (proamnion) anterior to it as was true in the Bird (Fig. 265). The only suggestion of this occurs much earlier in front of the beginning primitive streak sometime before the notochord has begun to develop (Fig. 262).

The Nature of the Mammalian Primitive Streak.— From the above description it is very evident that the parts here indicated are virtually homologous with the similarly named structures in the Bird. Consequently if the primitive streak of the latter can be further homologized with the remains of an elongated closed blastopore, it would appear that this homology holds equally well for the primitive streak of the Mammal. As previously suggested, however, because of practical difficulties experimental observations on the behavior of materials during and immediately after the formation of the primitive streak are not as yet available in this instance as they were in the Chick. The chief evidence therefore arises from observation of the relations of the streak to the formation of the notochord and mesoderm already noted, and to parts of the future embryo. Thus in the latter connection it may be stated that the anus forms at the posterior end of the streak, and a very marked pit, amounting in some cases to a virtual neurenteric canal, at its anterior end.

In the case of the preceding topic as in others to follow the student who does not recall the comparable situation in the Chick is again urged to refresh his memory on the points in question, since we shall not repeat identical material.

THE YOLK-SAC, THE ALLANTOIS, AND THE PLACENTA: THEIR STRUCTURE AND FUNCTIONS IN THE MAMMAL

Among the Amniotes of which the Chick is a type, i.e., the Birds, the chief organs through which the embryo receives its nutriment and effects respiration have been seen to be respectively the yolk-sac and the allantois. Among the vast majority of the Amniote group known as Mammals, however, these organs are very largely, and in many cases completely, supplanted in these functions by a new structure, typically associated with the *allantois* and termed the *placenta*. The large group of Mammals among whose members this organ is most fully developed is therefore known as that of the placental Mammals, a group which has already been frequently referred to. It will presently appear, however, that within this group there are certain types of placentas which vary from one another, both in their structure, and in the degree to

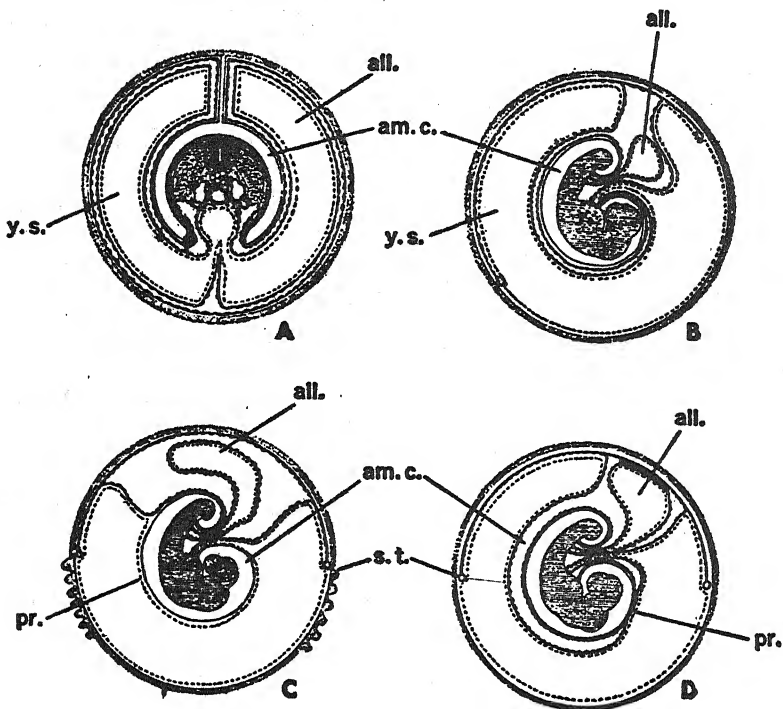


Fig. 269. — Fetal membranes of *A*, Monotremata; *B*, *C*, *D*, Marsupials. *B*. *Phalangista*, *Aepyprymnus*, *Didelphys*, *Bettongia*; *C*. *Dasyurus*; *D*. *Perameles* and *Halmaturus*. (In *Didelphys* the proamnion persists as in *Dasyurus*.) From Jenkinson (*Vertebrate Embryology*). (*A*, *B*, *D*, after Semon; *C*, after Hill.)

In this diagram of Mammalian fetal membranes the trophoblast (ectoderm of mammalian chorion) is stippled, the ectoderm of the amnion represented by a continuous line, the endoderm by a broken line, and the mesoderm (somatopleure and splanchnopleure) by a thick line swollen at intervals.

all. Allantois. *am.c.* Amniotic cavity. *pr.* Proamnion, i.e., portion of amnion without mesoderm. *y.s.* Yolk-sac. *s.t.* Sinus terminalis of area vasculosa.

which they have assumed the place and functions of the allantois and the yolk-sac. There exist also two relatively small mammalian groups, the *Monotremes* and the *Marsupials*, whose members possess either no placenta at all or only a very rudimentary one. Under these circumstances, therefore, it appears most convenient to treat the subject by taking up the conditions of the above organs in one group at a time. The *Monotremes* and the *Marsupials* will be considered first, since they are most primitive, and exhibit a condition most nearly akin to that in the *Reptiles* and *Birds*. After these there will be discussed certain orders of truly placental Mammals which best illustrate the various types

of allantoic placenta, and perhaps suggest its method of evolution. The orders to be thus considered are the *Ungulates*, the *Carnivores*, the *Rodents*, and the *Primates*. Finally before passing to a study of the first group, it may be mentioned incidentally that the discussion of this subject also necessarily involves in each case a more extended reference to the matter of implantation referred to above.

THE MONOTREMES

These curious mammalian forms comprise the Spiny Ant Eater (*Echidna*), and the Duck Bill (*Ornithorhynchus*). They are remarkable as Mammals in that they lay hard-shelled eggs like Birds. As might be expected in such a case, the yolk-sac is well developed and filled with yolk, while the allantois is also prominent. The placenta, on the other hand, because of its peculiar nature and functions, which its study will presently reveal, is naturally entirely lacking. In short, in eggs of this sort the embryonic parts under discussion are in all respects characteristically reptilian or avian (Fig. 269, A).

THE MARSUPIALS

This group comprises the Kangaroos (*Macropodidae*), the Opossums (*Didelphyidae*), the Marsupial Cats (*Dasyuridae*) and the Bandicoots (*Peramelidae*). These animals are all characterized by the fact that their young are born in a comparatively undeveloped condition. They then crawl inside of the Marsupial pouch of the mother and become attached to her teats, where they remain for some time. As might be expected under such circumstances, the means for obtaining nourishment and aerating the blood previous to birth are very primitive. In fact, among the various members of the group there occur some very excellent examples of graded transition from the condition in the Monotremes to that in the real placental Mammals. The Opossum is perhaps as primitive a form as any in this respect, and will therefore be considered first.

The Most Rudimentary Type of Placenta. — In *Didelphys*, or the Opossum (Fig. 269, B), the yolk-sac, as in all the Marsupials, is well developed though it contains no yolk. Nevertheless, upon its upper surface there is a clearly defined area vasculosa, bounded by a sinus terminalis. Since there is no yolk, however, the nutriment which the above area is to convey into the embryo must be obtained from some other source; this is accomplished in the following manner: Although

the mesoderm, and consequently the area vasculosa, do not reach to the opposite side of the yolk-sac, the endoderm on that side comes into contact with the trophoblast of the blastocyst. During implantation this trophoblast becomes thrown into folds (not shown in the figure) which fit into depressions in the uterine wall. The latter then secretes a viscid fluid, the uterine milk, which is absorbed via the trophoblast and endoderm, and finally reaches the embryo, partly at least by way of the area vasculosa. This contact of the embryonic trophoblast and the uterine tissue may be regarded as a very primitive beginning of what will later be recognized as a placenta. The allantois is very small in this case, as in most other Marsupials, and has no contact with the trophoblast. The exact means by which the embryonic blood is aerated, therefore, is a little uncertain. Very possibly, however, it also is accomplished through the contact of yolk-sac and maternal tissues.

A "Yolk-Sac Placenta." — *Dasyurus* is the second form to be considered, because it exemplifies the next step in the development of a true placenta (Fig. 269, C). The allantois, however, is still small, and the placenta-like structure which occurs is, therefore, again associated entirely with the yolk-sac. Furthermore, the trophoblast in contact with the non-vascular area of the sac once more forms the connection with the uterine wall. In this instance, however, this implantation is more thoroughgoing, and there appears for the first time that process of uterine erosion so noteworthy among some of the higher forms. This erosion is accomplished by the trophoblast which, after becoming thickened and syncytial (i.e., trophodermal) in certain regions, eats into the uterine epithelium and engulfs some of the maternal blood vessels. The blood so obtained passes in between the trophoblast and yolk-sac, secretions from one or both of which digest it so that it can be absorbed. Presumably also such an arrangement makes possible respiratory exchange of gases between embryonic and maternal blood. The type of contact which is here illustrated is so intimate that the area in which it occurs is sometimes referred to as a *yolk-sac placenta*.

A Primitive "Allantoic Placenta." — Finally, the most advanced condition in this Marsupial series is illustrated in *Perameles*, where the following situation occurs (Fig. 269, D): Here the yolk-sac is again large, and possesses an area vasculosa which is probably functional in absorbing some nourishment by way of the trophoblast. In this case, however, the allantois also is well developed, and comes into contact with the mesoderm of the chorion. Implantation then occurs and the trophoblast in the area of this contact becomes attached to the uterine

wall, whose epithelium in this region is transformed into a vascular syncytium. The trophoblast finally disappears, and the maternal blood vessels come into intimate contact with those which have grown out through the mesoderm of the allantois (Fig. 270). Thus there is established a true *allantoic placenta*. As will presently appear, however, the exact relationship of its embryonic and its maternal parts is different from that described in any of the subsequent types.

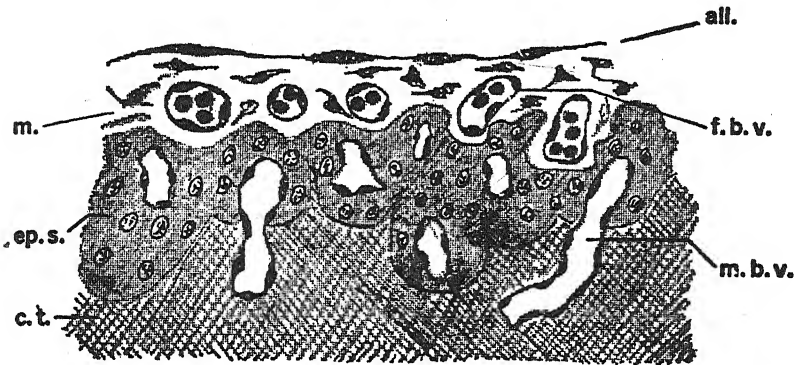


Fig. 270.—Section through the placenta of *Perameles*. From Jenkinson (*Vertebrate Embryology*). After Hill.

all. Allantoic epithelium. *m.* Mesoderm of allantois together with mesoderm of chorion. *f.b.v.* Fetal blood-vessel. *ep.s.* Syncytium of uterine epithelium. *m.b.v.* Maternal blood-vessels. *c.t.* Subepithelial connective tissue of uterus.

In connection with this, the first real placenta to be noted, there is one very important fact to be pointed out. Neither in this placenta nor in those of any other type does the fetal and the maternal blood actually mix. It is always completely separated by one or more membranes. Through these membranes, however, it is easily possible for an exchange of nutritive and waste materials, as well as gases, to take place.

This completes the account of the Marsupials, and we are now prepared to pass on to the orders of the genuine placental Mammals. As has been indicated, the latter are so named because here an allantoic placenta of one sort or another becomes the usual and chief means of embryonic nutrition and respiration. In the Marsupials, on the other hand, such a condition occurs only in the single instance last cited.

THE PLACENTARIA OR TRUE PLACENTAL MAMMALS

Within this large group, the embryonic appendages whose condition is being considered are probably in their most primitive form among

the Ungulates, and this order, therefore, will be treated first with special reference to the Mammal; we have selected for later detailed study, the Pig.

The Ungulates (the Pig).

The Early Means of Nutrition and the Yolk-Sac. — Before the blastocysts enter the horns of the bicornate uterus, the latter have been prepared for their reception during the pro-oestrus, oestrus and early

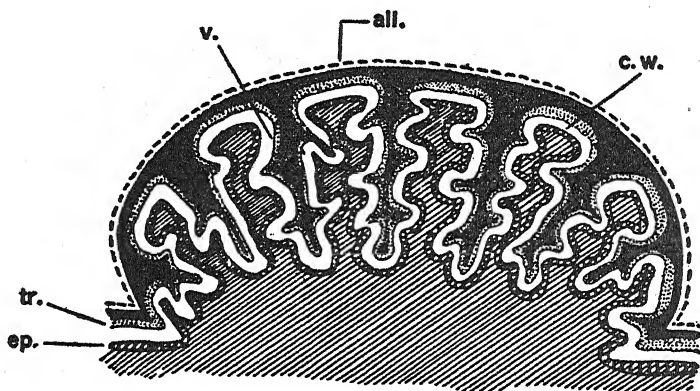


Fig. 271. — Diagram of a fetal and maternal cotyledon of the Cow. From Jenkinson (*Vertebrate Embryology*).

all. Allantoic epithelium. tr. Trophoblast. v. Villus. ep. Uterine epithelium continued into crypt. c.w. Wall of crypt. The maternal connective tissue is shaded.

dioestrus periods as explained in connection with the oestrus cycle. As a result of this the uterine walls are thickened, and their glands hypertrophied to produce the secretion (uterine milk) which helps to supply the embryos with nutriment and is eagerly absorbed by the trophoblast of the blastocysts. Meanwhile gastrulation has occurred, the endoderm (hypoblast) has grown around the inside of each blastocyst, and thus with the advent of mesoderm and the folding off of the gut, an empty yolk-sac is established in each. It is relatively large, and in the early stages possesses a well developed area vasculosa. Thus it is able to function actively in passing nutriment from the uterine cavity into the embryo. Later, however, the yolk-sac becomes insignificant, its function being entirely taken over by the allantois and the placenta, whose development will now be described.

The Placenta and the Allantois. — The blastocyst of this group, it will be remembered, soon becomes greatly elongated, reaching a length

of as much as a meter. It is not, however, to be understood from this that it is actually extended to this extent, for if it were it would be longer than the uterine horn in which it and several of its fellows are contained. Instead, as the threadlike blastocyst of the Pig grows, it becomes greatly folded, the folds fitting into corresponding folds of the

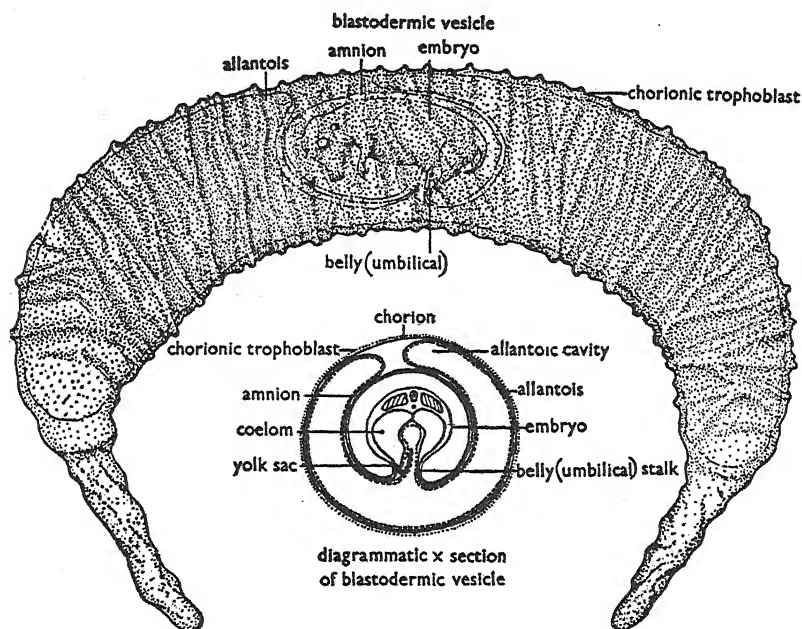


Fig. 272. — Drawing of a Pig blastodermic vesicle measuring about 350 mm. in length and 40 mm. in diameter, and a diagrammatic transverse section of same. The contained embryo measured about 40 mm. in length. Note the folds which replace the villi of many Ungulates.

uterine walls. Later when the embryo develops and the blastocyst expands, the latter is very much dilated and shortened, after which the term *blastodermic vesicle* is more commonly applied to it. As the vesicles reach their maximum length on about the thirteenth day, their trophoblast has become relatively adherent to the uterine epithelium, and *implantation* is said to have occurred.⁷ In the case of the Pig the surface of the endometrium remains folded as does the surface of the

⁷ The implantation time varies in different animals, but in most of them it occurs within a few days, often about seven, after the blastocysts reach the uterus. In a few cases, however, implantation may be markedly delayed. Thus in the Long Tailed Weasel and the Martin the blastocysts are said to lie dormant in the uterus for many weeks (Wright, '42).

blastocyst, though not to the extent that it was at its greatest length. This arrangement of course increases the area of trophoblastic and uterine contact through which the exchange of nutriment and excretory products can occur. This capacity for exchange is still further augmented by the fact that in certain spots (*areolae*) microscopic projections (*villi*) push out from the chorion into small spaces between the latter and the uterine epithelium. These spaces are filled with the uterine secretion referred to above. In some Ungulates such as the Cow, the villi

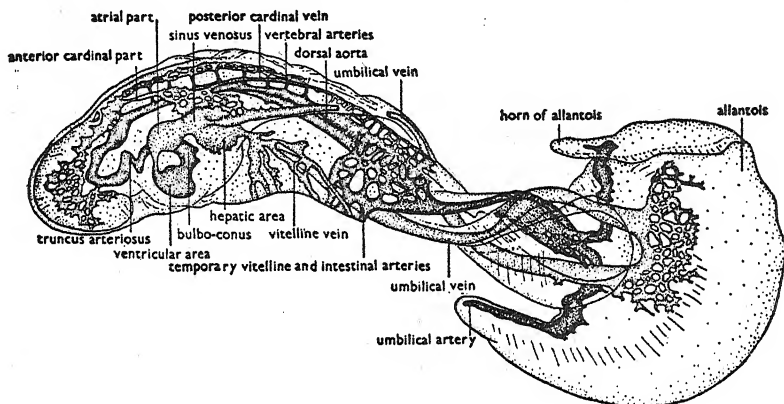


Fig. 273. — A 6.2 mm. Pig embryo (23 somites), injected, showing the circulatory system and beginning allantois. After Sabin.

are larger, and arranged in bunches or *cotyledons*, while the corresponding areas in the uterine wall with which the cotyledons come into contact are called *caruncles*. These latter are permanently located, and are said to exist as raised areas even in the uterus of the unborn calf. Thus in these instances the locations of the embryonic cotyledons are secondary, being determined by the positions of the maternal caruncles.

Meanwhile, to return to the Pig, by the time the embryo has reached a length of 4-6 mm. the allantois has begun to outstrip the yolk-sac, and soon comes to occupy the major part of the extra-embryonic space. It appears first as a rather conspicuous crescent-shaped outgrowth encircling the posterior of the embryo, with its horns extending anteriorly (Fig. 273). In this respect it differs considerably from the Chick allantois which it will be recalled is first noted as a roundish bladder pushing anteriorly and upward to the right from beneath the curled tail. The crescentic allantoic outgrowth of the Pig rapidly works its way around the amnion, pushes aside the now useless yolk-sac, and eventu-

ally extends everywhere throughout the extra-embryonic space of the vesicle except in the extreme ends (Fig. 272). The mesoderm which covers the allantois carries the umbilical blood vessels, and this mesoderm together with the capillaries of the vessels becomes closely adherent to the mesoderm of the chorion into which these capillaries penetrate. In this manner the fetal vessels come close enough to those of the uterine mucosa for the necessary exchanges to occur. Thus is constituted the Ungulate (in this case Pig) placenta, which as will be noted, comprises almost the whole surface of the blastodermic vesicle.

It is to be especially noted that in the processes just described there is absolutely no erosion of the uterine epithelium.⁸ Instead the chorionic folds simply fit in between those of the endometrium from which they may be easily stripped away at any time. Indeed during gestation the endometrium continues to secrete nutritive substances between itself and the chorion. This is absorbed by the latter and taken up by the embryonic vessels, so that in this case, as in some others, the embryonic nutriment is not all obtained directly from that which is carried in the maternal blood. A placenta in which the contact between fetal and maternal tissue is such as indicated is often defined as *indeciduate*. This term implies that at the time of parturition, the wall of the uterus is literally not deciduous. That is, there is no tearing away of maternal tissue when the fetal part of the placenta separates from that of the mother.

In concluding this discussion of implantation in the Pig a curious fact may be noted which apparently applies also to other Mammals which have two horned uteri and produce litters. Thus it is well known that the number of eggs ovulated by the two ovaries may be quite unequal as indicated by the corpora lutea present. Yet Corner has demonstrated that the number of embryos developing in each uterine horn is practically the same. This can only mean that enough of the embryos from the side which produced more eggs have migrated to the opposite side to equalize the numbers in the two horns. How this is brought about no one knows, but in the case of the Pig it apparently occurs previous to the elongation of the blastocysts.

The Carnivores.

The Yolk-Sac. — As in the Ungulates, the period of the pro-oestrus results in the accumulation within the uterine horns of a nutritive mix-

⁸ According to some authorities there is erosion of the maternal epithelium in the Ruminants.

ture somewhat similar to that already described. In some cases, however (e.g., the Cat), it appears to be less abundant than in the Ungulates, and of a more watery consistency. The uterine mucosa is of course also hypertrophied in the usual way, and everything is ready for the

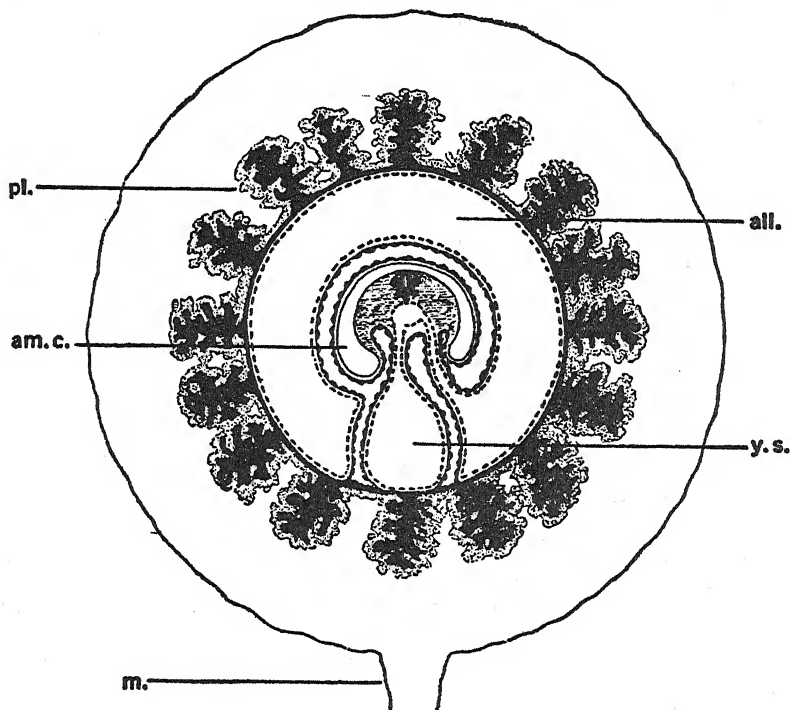


Fig. 274.—Fetal membranes and placenta of the Dog. From Jenkinson (*Vertebrate Embryology*). After Duval.

all. Allantois. *am.c.* Amniotic cavity. *m.* Mesometrium, or sheet of connective tissue attaching the uterus to the body wall. *pl.* Zonary placenta. (See text under description of the placenta of the Carnivores for the definition of this term.) *y.s.* Yolk-sac. The fetal mesoderm, connective tissue and blood-vessels are in black.

reception of the blastocyst, which in this instance is oval, never at any time threadlike. Again the latter begins its development by absorption of the nutrient fluid. A yolk-sac has meanwhile developed, in the usual Mammalian manner, and apparently it plays about the same part in this process as was noted in the Ungulates. As in that order, also, this appendage later becomes relatively insignificant (Fig. 274).

The Placenta and the Allantois.—While these events are occurring, a change is taking place in the uterine wall. In a band which completely encircles this wall the epithelium disappears. Likewise, in the

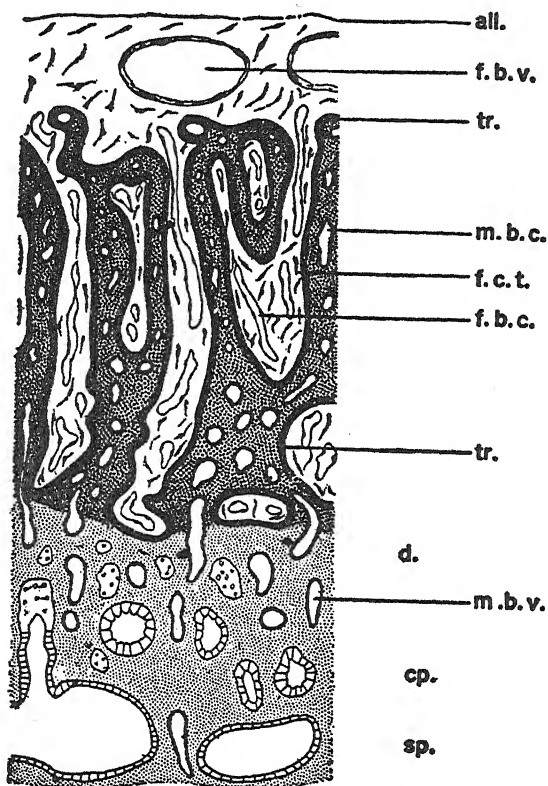


Fig. 275.—Section through the placenta and uterine wall of the Cat. From Jenkinson (*Vertebrate Embryology*).

all. Epithelium of allantois. *f.b.v.* Large fetal blood-vessels. *f.b.c.* Fetal capillaries. *f.c.t.* Fetal connective tissue. *tr.* Trophoblast (finely shaded). *m.b.c.* Maternal blood capillaries; these are immediately surrounded by maternal connective tissue (coarsely stippled). *m.b.v.* Maternal blood-vessels passing through the maternal glandular tissue (*d*). *cp.* Compacta (necks of glands). *sp.* Spongiosa (dilations of glands).

region of a corresponding band about the equator of the oval blastocysts, the latter begins to adhere to the prepared uterine wall. During this process of implantation, trophoblastic villi similar to those of some of the Ungulates begin to develop from the wall of the blastocyst in the region of its adherence. Because of the obvious band or zone-like shape of this region, the type of placenta which develops in this order is called *zonary*. The villi of the chorion, which may contain a core of

mesoderm, now push their way directly into the mucous tissue of the uterus. As they do so, they absorb any remaining epithelial debris which comes in their way. In this manner, they soon become firmly embedded in the maternal tissue and surrounded by maternal blood vessels. While this is going on, the allantois has grown out, and as in the Ungulates, soon becomes the chief appendage of the embryo. When the allantoic mesoderm comes into contact with the chorionic mesoderm in the zone of implantation, the allantoic capillaries penetrate the villi, and the placenta is virtually complete. During subsequent development, however, it becomes thickened somewhat by growth and branching of the villi and capillaries, and also of the maternal connective tissue in which they are embedded. The glands of the latter continue to supply debris and fat, which is absorbed by the chorionic villi up to the end of gestation. The main source of embryonic nutrition, however, is presumably material contained in the maternal blood (Fig. 275).

It will be noted that the attachment of the fetal and the maternal parts of the placenta is much more intimate in this case than it was in the Ungulates. This has resulted from the disappearance of the uterine epithelium, which allows the capillaries in the fetal villi to come that much nearer to those of the mother. Because of this very close attachment, it also happens that at birth a large portion of the maternal tissue is torn away with the fetal portion of the placenta. For this reason, this type of placenta may be regarded as *deciduate*. Indeed, as will appear from a study of the remaining groups, the Carnivores are probably the only animals possessing a placenta of which this is true in any large degree.

The Rodents.—As in the forms previously studied, the uterine epithelium of the horns is in a hypertrophied condition following the pro-oestrus and oestrus, and is thus ready to receive the blastocysts ("egg cylinders") when they reach the uteri. The method of attachment and of placenta formation which now follows varies somewhat in different Rodents, although it is fundamentally similar in all of them, and leads to practically the same results. It will further be found that in this case, the former process, i.e., attachment or implantation, is somewhat elaborate, and therefore requires more detailed attention than has hitherto been necessary. The chief conditions with respect to this process as well as to the general character of the yolk-sac, may be illustrated by reference to two forms, the Mouse and the Rabbit.

Implantation and the Development of the Yolk-Sac.—In the case of the Mouse, each elongated uterine horn becomes lined with pits upon its *anti-mesometric* side. This is the side opposite its point of attachment to

the coelomic wall, the latter region being termed the *mesometric* side. Each of the ovoid blastocysts, of which there are several in the Mouse, becomes embedded in one of these pits with the embryonic knob facing the narrow lumen of the uterus (Fig. 276, *B*). That this anti-mesometric

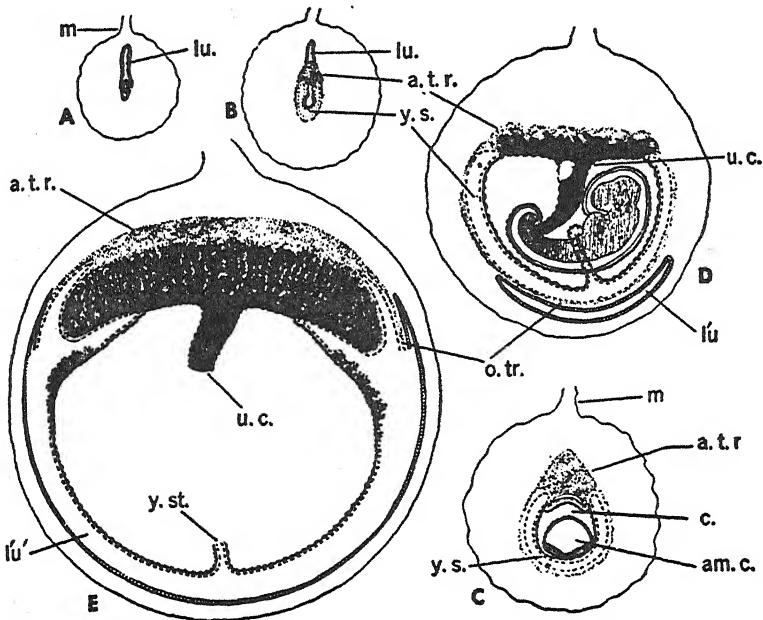


Fig. 276. — Five stages in the formation of the placenta in the Mouse. From Jenkinson (*Vertebrate Embryology*). *A*. The blastocyst free in the uterus. *B*. The blastocyst attached and the placental thickening of the developed allantoidean trophoblast (trophoderm) (*a.t.r.*). *C*. Later stage, after closure of the amniotic cavity (*am.c.*) and the obliteration of the uterine lumen. *D*. Placenta becoming established, and reappearance of uterine lumen (*l'u.*). *E*. Elaboration of the placenta. Disappearance of the distal wall of the yolk-sac and omphaloidean trophoblast (*o.tr.*).

c. Extra-embryonic coelom. *l'u.* New uterine lumen on the anti-mesometric side. *lu.* Original lumen of the uterus. *y.s.* Yolk-sac. *y.st.* Yolk-stalk. *u.c.* Umbilical cord. *m.* Mesometrium.

implantation is not the result of gravity has been clearly demonstrated in the Rat by Alden ('45). He cut out the middle portion of a uterine horn, leaving blood vessels intact, and replaced it in an inverted position. Implantation in this section was still on the anti-mesometric, but now dorsal, side. Continuing with the case of the Mouse the further history of a single blastocyst will suffice.

As soon as the embedding has occurred, the trophoblast immediately starts to erode the epithelium of the pit, and to devour the debris which

results. Meantime the blastocyst enlarges sufficiently so that the side containing the embryonic knob crosses the uterine lumen and comes in contact with the opposite wall (Fig. 276, B, C). In this way, each blastocyst obtains attachment at every point, and completely obliterates the cavity of the uterus where it is situated. At every place where contact is thus

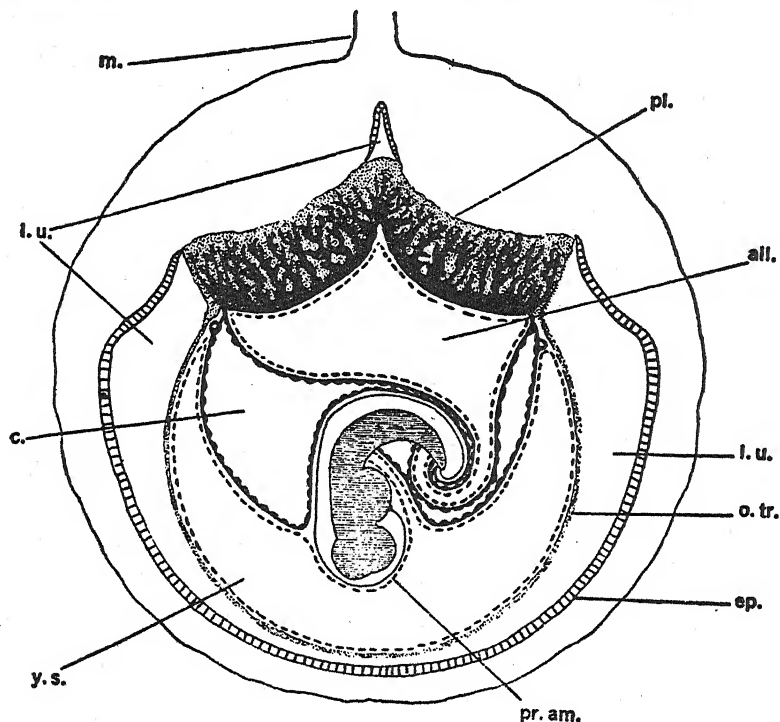


Fig. 277. — Fetal membranes and placenta of the Rabbit. From Jenkinson (*Vertebrate Embryology*). After Duval and Van Beneden.
pr.am. Proamnion. Other letters as in Fig. 276.

established, i.e., on the bottom and sides of the original pit, and also upon the uterine wall opposite to it, erosion of the uterine epithelium is carried on. The placenta, which will presently be described, is established on the mesometric side of the uterus at the second point of contact, and therefore next to the embryo. Then, owing to the intimate relation of trophoblast and allantois in this region, the thickened trophoblast (*trophoderm*) on this side of the blastocyst is called *allantoidean*. On the opposite side, i.e., at the original bottom of the pit, the uterine lumen is later again established. Here for a while epithelium once more develops, and covers both the wall of the uterus and the blastocyst (Fig.

276, D). Inside the latter, the yolk-sac has meanwhile formed, and on its upper surface has acquired an area vasculosa. Its lower wall, on the other hand, which is in contact with the trophoblast of the blastocyst, finally degenerates. The trophoblast (in this region termed *omphaloid-ean*) and the newly formed epithelium at this point then also vanish, and thus the interior of the yolk-sac is placed in immediate communication with the re-established uterine cavity (Fig. 276, E).⁹

Turning now to the method of implantation in the Rabbit, it is found to be somewhat less complicated. Here a pair of folds arise upon the mesometric side of the uterus, and the blastocysts become attached to these. Each blastocyst in this case lies between the folds and becomes attached by the trophoblast on either side of the embryonic disc. In these regions, the uterine epithelium is eroded, and two placentas are established which later merge into one (Fig. 277). The opposite side of the blastocyst forms no intimate contact with the uterine wall and presently disappears. Concurrently the ventral wall of the yolk-sac also disappears, so that again, as in the case of the Mouse, the cavity of the sac is directly continuous with that of the uterus (this stage not shown in the figure).

Having thus described the two chief types of implantation among the Rodents, we are now in a position to discuss the nature of the placenta and other means of nutrition common to all this group.

The Placenta and the Allantois. — During the erosion of the uterine epithelium indicated above, the allantoidean or placental trophoblast becomes greatly thickened, to form trophoderm. This trophoderm then continues to eat down into the mucous layer of the uterine wall, engulfing, as it does so, maternal blood vessels, together with glycogen from the glycogen-filled cells (*maternal glycogen tissue*). There next appear in the trophoderm numerous *lacunae*, and into these is emptied the maternal blood from the vessels whose walls have been destroyed (Fig. 278, A). Meantime an allantois has arisen. In the Rodents, the endodermal portion of this organ containing the cavity is usually small, although in the Rabbit, which in this as in most other respects is more primitive, the allantoic cavity attains a considerable size (Fig. 277). The mesodermal part, however, is always well developed, and soon reaches the trophoderm of the placental region, bringing with it the umbilical blood vessels (Fig. 278, B). The capillaries of these vessels then

⁹ The assumption has been that in this as in other cases the vascularized wall of the empty yolk-sac functions in obtaining nutriment for the early embryo. Recent experiments on the Rat, however, involving the tying off of the vitelline vessels, seem to indicate that such a function is negligible, at least in this animal (Noer '47).

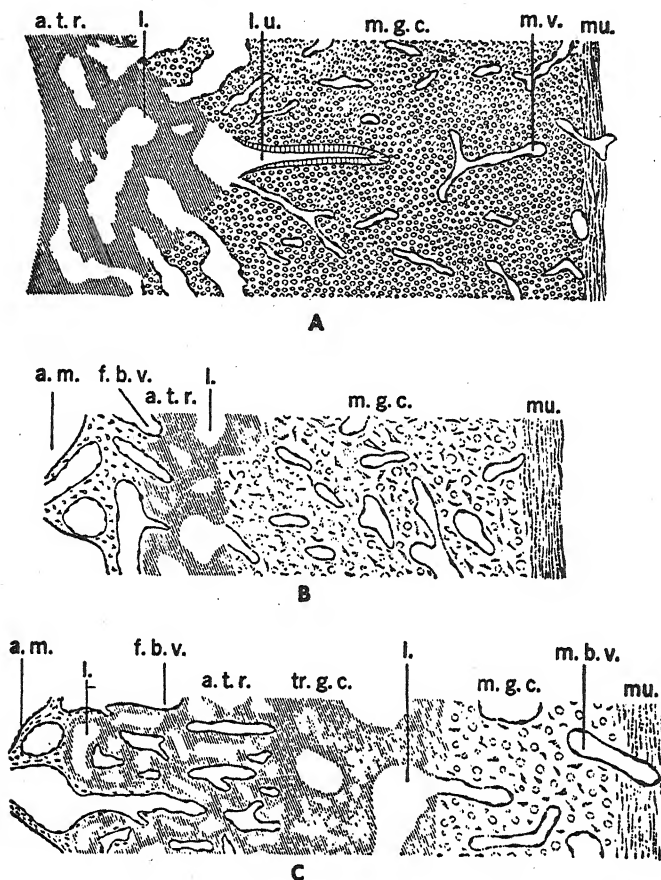


Fig. 278. — Placentation of the Mouse. Details of the five stages of Fig. 276. From Jenkinson (*Vertebrate Embryology*).

A. Strip of a section through the allantoidean trophoblast (trophoderm) and overlying maternal tissues in stage C, Fig. 276.

a.t.r. Allantoidean trophoderm. mu. Muscularis. m.v. Maternal blood-vessel, opening below into l. lacunae of the trophoderm. l.u. Original lumen of the uterus. m.g.c. Maternal glycogen tissue.

B. Similar strip of the same parts in stage D, Fig. 276.

f.b.v. Fetal blood-vessel. a.m. Allantoic mesoderm. Other letters as in A.

C. Similar strip of the last stage, Fig. 276.

tr.g.c. Trophodermal glycogen tissue. Other letters as in B.

Note that ultimately this placenta is very largely composed of trophoderm, which is a non-maternal tissue. Hence, since at parturition the line of separation passes through the placenta (the trophodermal glycogen tissue), little or no maternal tissue is lost, and the placenta is essentially indeciduate. (See text.)

penetrate the trophoderm so as to come near to the cavities containing the extravasated maternal blood. This blood is being constantly poured into the central space of the placental region, and withdrawn at the periphery through the maternal veins. Gradually, toward the maternal side, the trophoderm surrounding the lacunae becomes further vacuolated through the secretion of glycogen, thus establishing a *trophodermal glycogen tissue* (Fig. 278, C). Eventually through the increase of the latter, the layer of original maternal glycogen tissue is entirely eliminated.¹⁰ Such is the character of the completed placenta of the Rodents, which, because of its development upon only one side of the blastocyst, has the general shape of a disc or button. It is, therefore, termed *discoidal*, as distinguished from the zonary form found in the Carnivores.

Comparing the placenta in this case with that noted in the Carnivores, the chief difference will be found to be that, in the completed organ of the Rodents, maternal tissue plays very little part. The placenta indeed is principally composed of the fetal trophoderm with its capillaries, lacunae, and glycogen tissue. This difference seems to be achieved by the fact that the trophoderm erodes not only the uterine epithelium, but a large part of the mucosa and its blood vessels as well. Because of this peculiar structure, it happens at parturition that, aside from the blood in the lacunae, very little real maternal tissue is lost. This follows from the fact that the actual line of separation runs through the region of vacuolated cells which have now lost their glycogen and collapsed, and this region, as noted, is held to be entirely trophodermal. On account of this lack of maternal tissue to be torn away, many authorities regard the term deciduate as a misnomer when applied to placentas of this type. If the above description be correct, it apparently is a misnomer. Nevertheless, such placentas are still commonly classified under this head.

As regards the method of nutrition in this order, it is apparent that, aside from the glycogen, nutriment is chiefly obtained, so far as the placenta is concerned, from the maternal blood. It will be remembered, however, that among the Rodents, the yolk-sac is always eventually open to the uterine cavity. Thus, for instance in the Mouse and the Rabbit, the lower epithelial wall of this organ was found to disappear completely, while in the Guinea Pig it is never even formed. This being the case, the upper wall of the sac may, in some cases at least, function throughout gestation in the absorption of uterine secretions. To the ex-

¹⁰ The maternal glycogen tissue is said to be more abundant and persistent in the Rabbit.

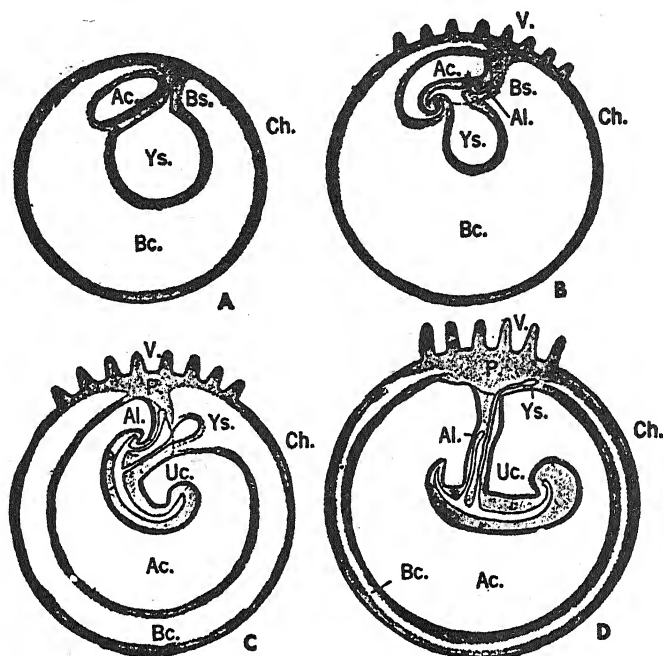


Fig. 279. — Diagrams illustrating the formation of the umbilical cord and the relations of the allantois and yolk-sac in the Human embryo. From McMurrich (*Development of the Human Body*). The heavy black line represents the embryonic ectoderm; the dotted line marks the line of the transition of the body (embryonic) ectoderm into that of the amnion. Shaded areas, mesoderm.

Ac. Amniotic cavity. Al. Allantois. Bs. Body-stalk. Ch. Chorion. P. Placenta. Uc. Umbilical cord. V. Chorionic (trophodermal) villi. Ys. Yolk-sac.

tent that this is true, therefore, the Rodent yolk-sac, both in its form and in its activity, differs markedly from the types previously studied within the strictly placental group.

The Primates.¹¹

The Allantois and the Yolk-Sac. — In the order of Primates, the nature of the yolk-sac and allantois is somewhat unique, while the latter

¹¹ The characteristics of the embryonic appendages which are ascribed to this order apply to only one of the family of *Lemurs*, i.e., *Tarsius*. This animal, in respect to these organs, may be classed with the lower Monkeys. So far as is known, however, all other Lemurs are similar to the Ungulates as regards the yolk-sac and allantois, and also even in the possession of a diffuse indeciduate placenta. This exception must be borne in mind with reference to all statements concerning the Primates as a whole.

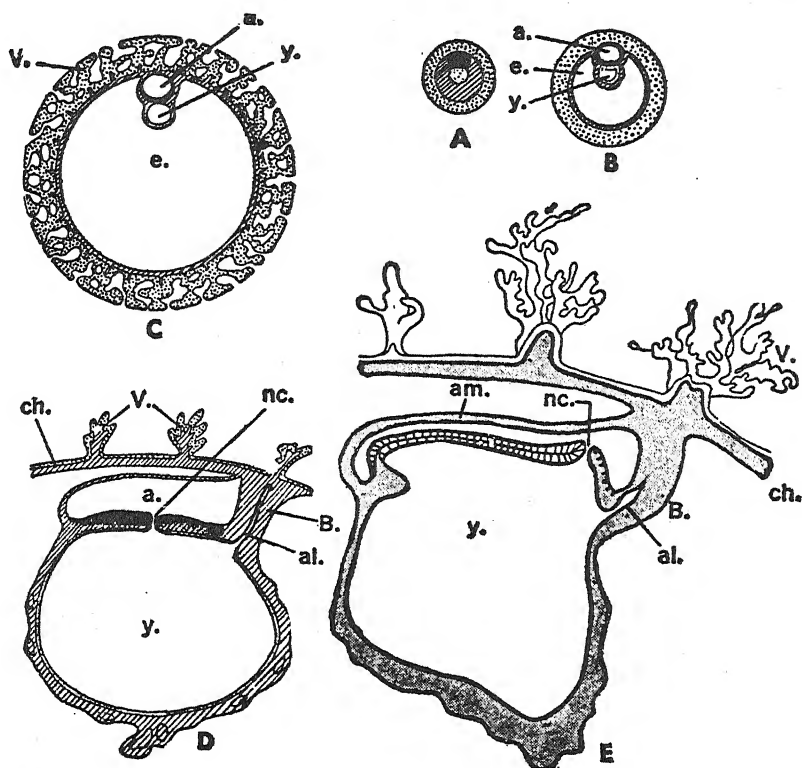


Fig. 280. — Diagrams of sagittal sections through the Human blastodermic vesicle, showing the formation of the amnion and trophoderm. From Kellicott (*Chordate Development*). A-D, after Keibel and Elze. E. From McMurrich (*Development of the Human Body*), after Graf von Spee. In all the figures the anterior end is toward the left, and in all the figures except E the following conventions are used: Black, embryonic ectoderm; heavy stipples, trophoblast and trophoderm; light stipples, endoderm. Oblique ruling, mesoderm except in A. A. Hypothetical early stage; oblique ruling represents magma reticulare (see text). B. Amniotic cavity and wide exocoelom established; endoderm limited to a small vesicle beneath the embryonic ectoderm. The exocoelom in reality contains scattered mesenchyme cells. C. Blastodermic vesicle enlarged and covered with trophodermal villi, into which the mesoderm is extending. Endodermic vesicle (yolk-sac) very small (stage of Peter's ovum). D. Embryonic portion only, of an older vesicle showing the neurenteric canal, primitive streak (in the plane of the section posterior to canal), and body-stalk. The mesoderm of the yolk-sac is becoming vascular. E. Sagittal section through a Human embryo of 1.54 mm. (Graf von Spee's embryo Gle).

a. Amniotic cavity. al. Allantois. am. Amnion. B. Body-stalk* (umbilical cord). ch. Chorion. e. Exocoelom. nc. Neurenteric canal. V. Chorionic villi. Y. Yolk-sac.

organ is also peculiar in its method of development. An account of these structures will be given, therefore, before proceeding to the matter of implantation and placenta formation within this group.

First, as regards the allantois, it will be found that the endodermal sac is even more limited than it was in the majority of the Rodents. Furthermore, the mesoderm of that organ does not comprise, as in most

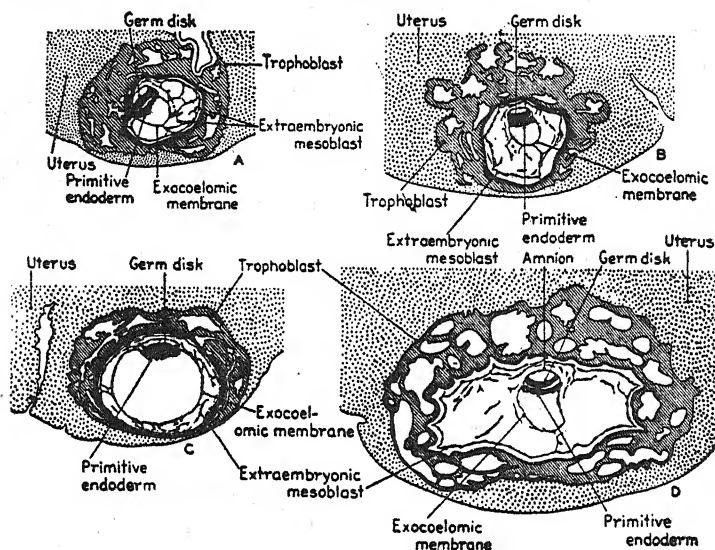


Fig. 281. — Mid-sagittal sections through four Human blastocysts ("ova") and surrounding uterine wall. After Hertig and Rock. *A* and *B* are estimated as 11 days old plus, while *C* and *D* are estimated as 12 days old plus. *B* is the Miller "ovum," while *D* is the Werner (Stieve) "ovum."

previous cases, a mere covering for the sac; instead, it forms a thick stalk, the *body-stalk*, or *umbilical cord*, which attaches the embryo to the chorion or wall of the blastocyst. Into the proximal end of the mesodermal cord, the hollow endodermal element then projects for only a short distance (Figs. 279 and 280). This condition is brought about as follows:

From what is known of the earliest human embryos (7–15 days, see below) the blastocyst, following cleavage and gastrulation, contains the following structures and materials. First there is the blastoderm, consisting of a layer of ectoderm and endoderm with a small amniotic cavity derived apparently from a split in the embryonic knob (Method II, Type *b*, see above). Second, the greater part of the blastocoelic space is

occupied by a reticulate material, the *magma reticulare*, which probably consists of coagulated protein containing fluid. Scattered through this reticulate substance, and lining parts of the trophoblast, are a few mesoderm cells (extraembryonic mesoblast) presumably derived from the blastoderm (Fig. 281, *A*, *B*). At about the center of the blastocyst in these human specimens there occurs a particularly definite space bounded laterally and ventrally by an especially clearly defined layer of the reticulum, termed the *exocoelomic membrane* or *Heuser's mem-*

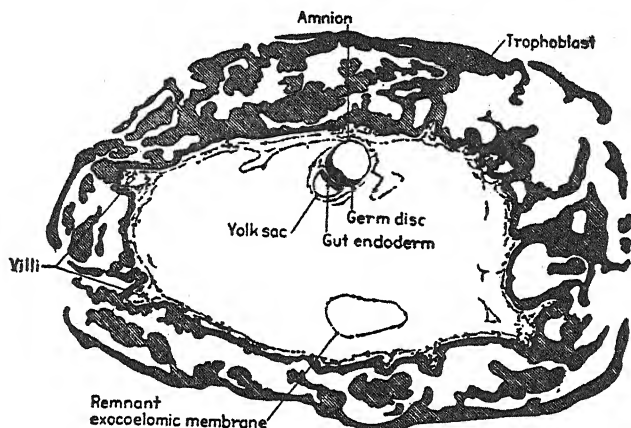


Fig. 282. — Mid-sagittal section of a Human blastocyst and surrounding uterine wall with an estimated age of 15 days, the Edwards-Jones-Brewer "ovum." After Hertig and Rock.

brane (Fig. 281). Dorsally this space is lined by the endoderm of the blastoderm, and it has therefore been interpreted by some as the yolk-sac. Others maintain that the true yolk-sac does not appear until slightly later, about the 13th day. It is difficult, however, to distinguish the "endoderm" of this later yolk-sac from the exocoelomic membrane bounding the central "exocoelomic space" of the earlier embryos. At all events in these later stages the *magma reticulare* has mostly disappeared and the trophoblast is lined by a definite layer of mesoderm. This also extends around what is now termed the yolk-sac, up over the amnion, and at what proves to be the posterior end of the embryo, serves to attach the blastoderm to the trophoblast (Figs. 280, *D*; 281, *D*; 282). This mesodermal attachment later comes to constitute the umbilical stalk already referred to, and into it there presently grows a small out-pushing from one side of the sac where the latter joins the blastoderm. It is the beginning of the very small allantois (Figs. 279, 280, *D*, *E*).

Although at first located somewhat dorsally, the embryonic end of the stalk soon moves around so as to be attached to the embryo on its ventral side. It retains, however, its original point of attachment to the chorion since it is here that the placenta is to be formed.¹² From this description it is evident that in the Primates, the allantois, or more strictly in this case, the umbilical cord, does not grow out from the embryo to the trophoblast. It is there from the first.¹³

As concerns the yolk-sac, it is only necessary to state that it is very rudimentary, having little or no function. The space which might otherwise be occupied by these appendages, however, is eventually filled in this order by a very large amnion.¹⁴

Implantation and Placenta Formation.— According to previous accounts ovulation occurs following what amounts to a pro-oestral uterine hypertrophy, and the blastocyst reaches the uterus while the latter is under the influence of the progesterone of the succeeding corpus luteum. Here implantation takes place through the erosion of the hypertrophied endometrium by the newly arrived blastocyst between one or two weeks following ovulation. This is of course previous to the time of the menstruation which would have occurred had pregnancy not intervened.

As in the case of the Rodents the details of the implantation process vary somewhat. In this instance, the chief variation occurs, so far as is known, between two groups, i.e., Tarsius, together with the other lower Monkeys, and the higher Apes, together with Man.

As regards the first group, i.e., that of Tarsius and the Monkeys, the description may be brief. The region of implantation may occur on the dorsal or ventral wall of the uterus, depending upon the form in question, and is not marked by either pits or folds, as in the Rodents. When

¹² In Tarsius the placenta is formed on the opposite side of the blastocyst, and the stalk shifts its point of attachment to the trophoblast accordingly.

¹³ In a more recent human specimen, the Martin-Falkiner blastocyst ('38), estimated at seventeen days of age, a somewhat different theory is expressed concerning the development of these structures. These investigators seem to think that both the yolk-sac and allantois may arise as vesicles developing in the inner cell mass itself, and that they may later all run together. If this is true it involves a somewhat novel method of gastrulation, and a peculiar fate for the allantois. Since there is some question about the normality of this embryo, theories based on it should await confirmation from the study of more specimens.

¹⁴ Though not certainly known, it appears that the amnion in the Primates (excepting the Lemurs, in this instance including Tarsius) is formed in a manner similar to that described under method II, i.e., by the development of a cavity in the embryonic knob. The process in this group differs from that described under types *b* or *c* of the second method, however, in that in this case the embryonic knob does not move down to the opposite side of the blastocyst.

the trophoblast of the blastocyst comes into contact with the hypertrophied uterine endometrium it promptly erodes the epithelium. A discoidal placenta which is very similar, if not identical, with that described for the Rodent, then develops at the place in question. Later, a

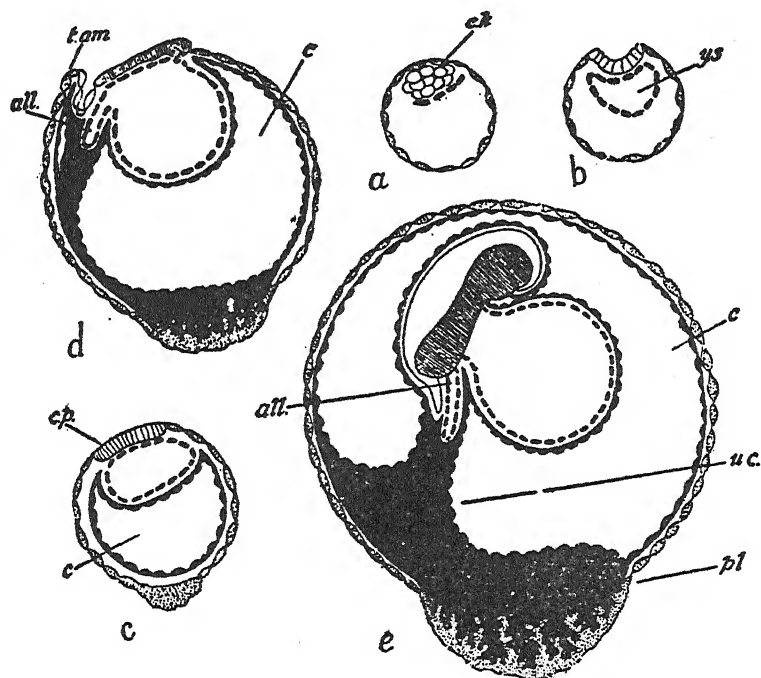


Fig. 283. — Development of the fetal membranes in *Tarsius*. From Jenkinson (*Vertebrate Embryology*). After Hubrecht.

a. Blastocyst before Rauber's cells have disappeared. b. The embryonic knob (e.k.) is being folded out to the surface; the yolk-sac is complete. c. The embryonic plate (c.p.) is at the surface, the extra-embryonic coelom (c) is formed. d. The tail fold of the amnion is growing forward (t.am.), the allantois (all.) has penetrated the mesoderm of the body-stalk, a placental thickening has been developed at the anti-embryonic pole. e. The amnion is closed and the body-stalk or umbilical cord (u.c.) is shifting its position, to be attached to the placenta (pl.).

second similarly shaped placenta may form where the blastocyst comes in contact with the opposite side of the uterus. The umbilical cord, of course, reaches only one of these, but the two are connected by blood vessels (Fig. 283, only one placenta in this case).

Considering now the second group, i.e., the higher Apes and Man, it unfortunately happens that as regards the earliest stages relatively little is definitely known, chiefly because of the scarcity of material. Some of

the earlier classic cases which have been studied comprise the Miller blastocyst Streeter ('26) with an estimated age of 11 days and a diameter of 0.4 mm., the Bryce-Teacher blastocyst, estimated age 12-14 days, diameter 0.64 mm., and the Peters blastocyst, estimated age 14-15 days, diameter 1.1 mm.¹⁵ Somewhat more recently others have been added to

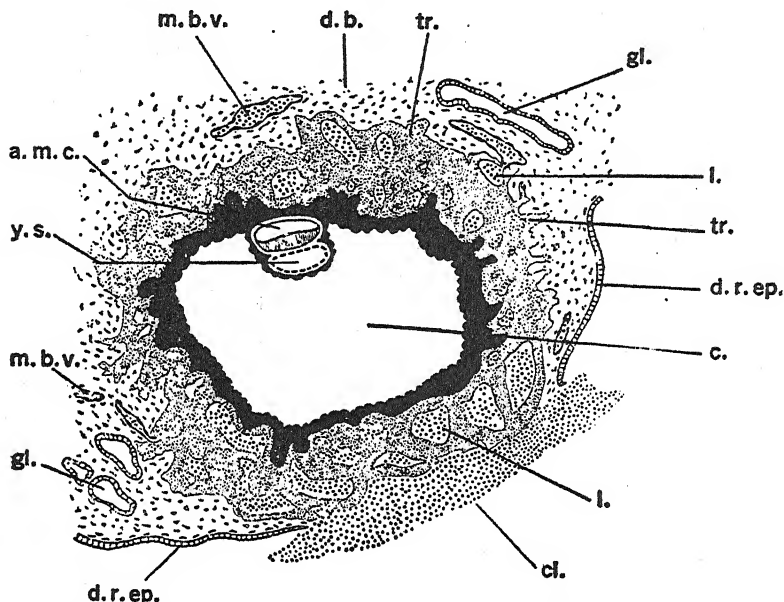


Fig. 284. — Early Human embryo with its membranes. From Jenkinson (*Vertebrate Embryology*). After Peters.

am.c. Amniotic cavity. *c.* Extra-embryonic coelom. *d.b.* Decidua basalis (serotina). *d.r.ep.* Uterine epithelium covering the decidua reflexa or capsularis. *l.* Lacuna in trophoblast (*tr.*). *gl.* Uterine gland. *m.b.v.* Maternal blood-vessels opening here and there into lacunae. *cl.* Clot marking (probably) the point of entrance of the blastocyst; here the uterine epithelium is interrupted. *y.s.* Yolk-sac.

the list, all of about the same or slightly greater estimated age. Thus there is the Werner (Stieve) blastocyst at 12 days, and the Edward-Jones-Brewer blastocyst (Brewer, '37) at 15 days with internal dimensions of 1.85 x 1.71 x 1.01 mm., and the previously mentioned Martin-Falkner blastocyst, estimated age 17 days with possible abnormalities. Latest of all, are the Hertig-Rock blastocysts, one of which (not shown in the figures) is estimated at about 7 days, the youngest yet dis-

¹⁵ Whether some of these specimens have quite reached the blastocyst stage is perhaps open to question, but they are certainly not "ova" as they have sometimes been designated.

covered (Hertig and Rock, '41; Figs. 281, 282). The additional data from all the clearly normal sources, however, has not substantially modified the conclusions previously held concerning the early stages already described, and the processes about to be discussed. From information obtained from these early specimens, and from conditions which are known to exist later on, implantation and development both in Man and the higher Apes is thought to be as follows:

The blastocyst usually becomes attached to the dorsal (i.e., posterior) wall of the uterus in Man, and to the ventral (i.e., anterior) wall in the Apes; here the trophoblast promptly starts its work of erosion. In this case, however, the process goes much further than in the instances so far noted. In fact, it is thought that by this means the blastocyst becomes completely buried in the mucous layer of the uterus, while the epithelium closes behind it. It thus virtually occupies the position of an internal parasite within the uterine tissue (Fig. 284). As growth now proceeds, the blastocyst, covered by a layer of uterine mucosa and some epithelium, begins to project into the cavity of the uterus. Meanwhile, it appears that changes are taking place in the trophoblast, or chorion, as it may be called, quite similar to those which occurred in the Rodent, i.e., a thickening, and the formation of lacunae. In this case, these processes by which the trophoblast is thus converted into the trophoderm at first occur on every side of the blastocyst. Presently, however, the trophodermal development becomes much more marked on the inner side, i.e., that side away from the cavity of the uterus, and it is here that the permanent discoidal placenta is soon formed.

Throughout the trophoblast or chorion (now trophoderm) but especially on the placental side, the embryonic blood vessels, surrounded by a sheet of connective tissue (chorionic mesoderm), are working their way among the lacunae, into some of which they project. These vessels and their connective tissue are covered with a thin trophodermal cell layer known in human embryology as the *cell layer of Langhans*. Outside of this, there is an added layer of the trophoderm which is syncytial, and is apparently derived from the cells of Langhans, the latter being gradually used up. Thus, where the blood vessels, pushing their trophodermal and mesodermal layers before them, project into the lacunae, they have something like the appearance of villi, and are often so referred to (Fig. 285). It should be clearly understood, however, that these "villi" are in no sense homologous with the true villi described in connection with the indeciduate placenta of the Ungulates. They are not indeed essentially different from the capillaries which push into,

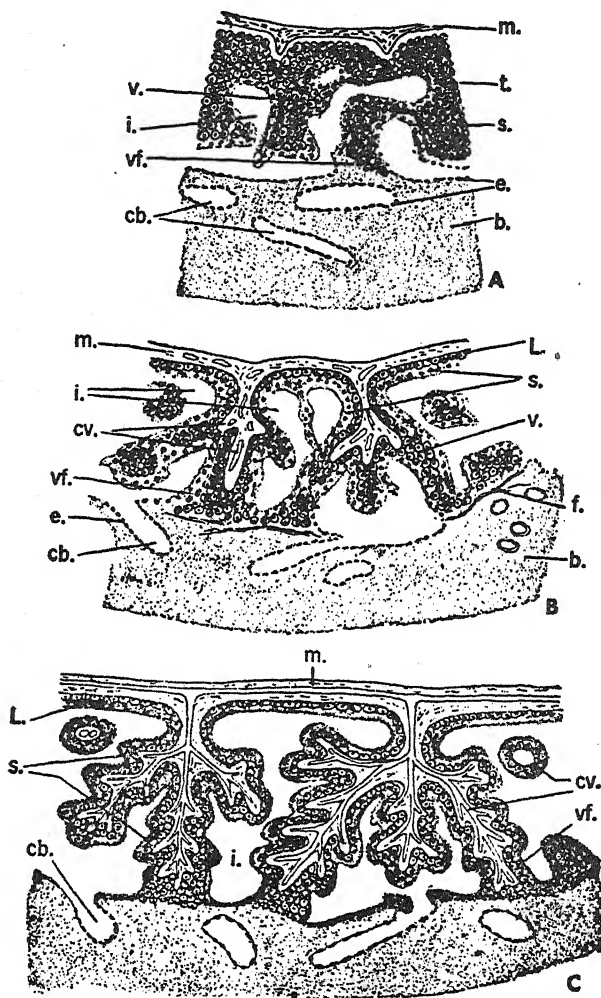


Fig. 285. — Diagrams illustrating the development of the "villi" in the Human placenta. From Kellicott (*Chordate Development*). A, B. After Peters. C. After Bryce. A. Chorionic mesoderm just beginning to extend into the villi. B. Mesoderm invading the villi which are now branched. Layer of Langhans cells forming beneath the syncytiotrophoderm. C. Continued branching of the villi, all now covered only by the syncytiotrophoderm and the single layer of Langhans cells.

b. Decidua basalis. cb. Capillaries of the decidua basalis. cv. Capillaries of the villi. e. Endothelium of the maternal capillaries. f. Fibrin deposited at the junction of the trophoderm and decidua basalis. i. Intervillous cavity (i.e., lacuna or sinus) filled with maternal blood. L. Langhans' cells. m. Chorionic mesoderm. s. Syncytiotrophoderm. t. Trophoderm. v. Villi. vf. Fixation villi, i.e., those which extend clear across a sinus.

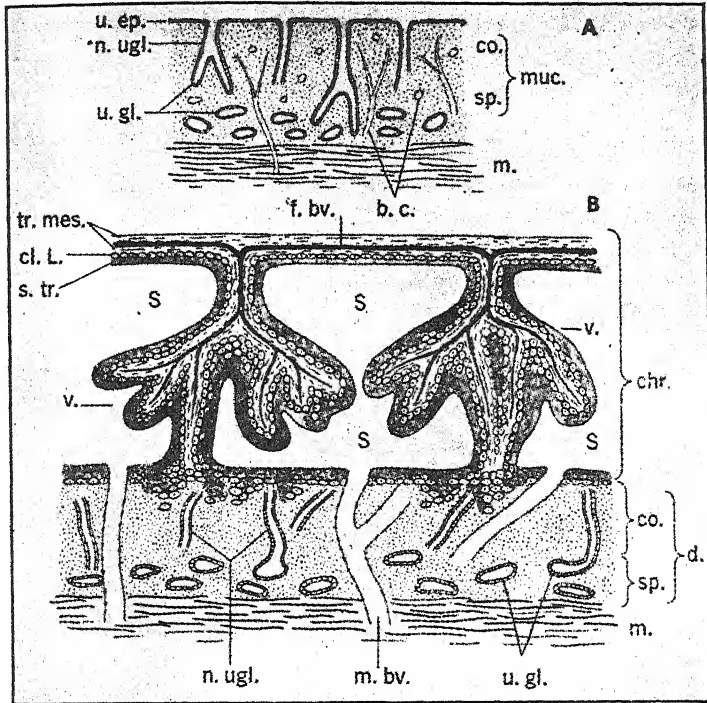


Fig. 286. — A. A diagram of an idealized section through the inner portion of the wall of the non-pregnant uterus a short time previous to the beginning of menstruation. The muscular layer is very thick, and only a small portion of it is shown. Beyond this layer on the outside of the uterus would come the peritoneal covering or serous membrane which here as elsewhere is quite thin. B. A diagram of a similar section through the Human placenta at a slightly later stage than that shown in Fig. 285 (according to Jenkinson). The trophoderm, it will be noted, has penetrated slightly into the compacta in this stage, so that the "villi" are more firmly attached. Note that these "villi" are quite different in their relation to the maternal tissue from that observed in the Ungulates, (Compare Fig. 271). No attempt has been made to distinguish between afferent and efferent blood vessels, although it is to be understood that both types exist on both the embryonic and maternal sides.

bc. Blood capillaries in the mucosa. *c.L.L.* Cell layer of Langhans, still clearly in evidence. *Chr.* Chorion consisting of trophoderm plus extra-embryonic mesoderm. *co.* Compacta. *d.* Decidua; for explanation of terms see further in text. *f.bv.* Fetal blood vessels. *m.* Muscular layer of uterus, or muscularis, only a small portion of which is shown. *mbv.* Maternal blood vessels. *n.ugl.* Necks of uterine glands in the compacta. *s.* Sinus lined by syncytial trophoderm, and filled with maternal blood. That the syncytial layer and cells of Langhans line the sinuses on the side of the decidua is questioned by some authors. *sp.* Spongiosa. *str.* Syncytial trophoderm. *tr.mes.* Trophodermal (chorionic) mesoderm. *u.ep.* Uterine epithelium. *u.gl.* Uterine glands. *v.* "Villus."

and are hence covered by, the trophodermal material in the Mouse or Rabbit. As regards the lacunae, they are again filled with maternal blood, and are often termed "sinuses." They also are lined by a syncytial layer of the trophoderm augmented to some extent by a layer of the cells of Langhans, similar to, and continuous with, that which covers the connective tissue of the fetal capillaries (Jenkinson).

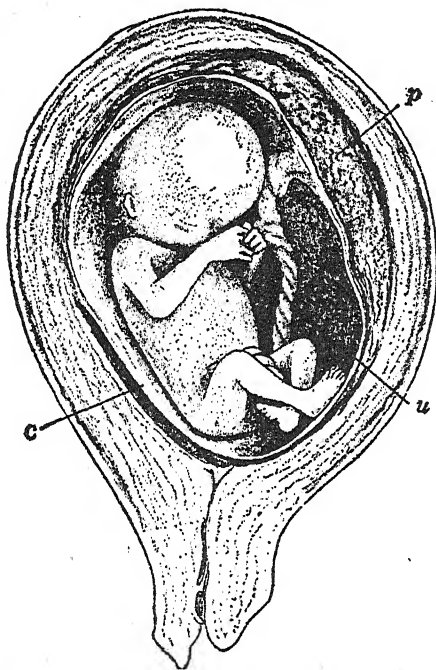


Fig. 287. — Human embryo of the fourth month *in utero*, showing the arrangement of the membranes and placenta. From Kellicott (*Chordate Development*). After Strahl.

c. Chorion and amnion. p. Placenta. u. Umbilical cord.

Outside of the discoidal placental region, the whole blastocyst is growing out so as to fill the cavity of the uterus (Figs. 287 and 288). Its wall in this area consists internally of extra-embryonic mesoderm, and externally of the trophoderm, the two together as usual constituting the chorion, while within this chorionic trophoderm the "villi" and lacunae are only slightly developed. Lastly, tightly adherent to, and covering this trophoderm, comes the uterine mucosa and epithelium which covered the blastocyst after its embedding in the uterine wall. As growth continues, this epithelium is eventually bound to come in contact with that which lines the walls of the uterus at other

points. By the time this occurs, however, the uterine epithelium and mucosa covering the growing blastocyst has become distended and is disappearing. Thus the trophoderm of this region is brought into direct relations with the epithelium which elsewhere still remains on the walls of the uterus, and this epithelium too presently disappears. Concurrent with the complete filling of the uterus and the disappearance of all its epithelium the chorionic layer of the blastocyst is everywhere united to the sub-epithelial mucosa of the uterine wall. It is only in the region

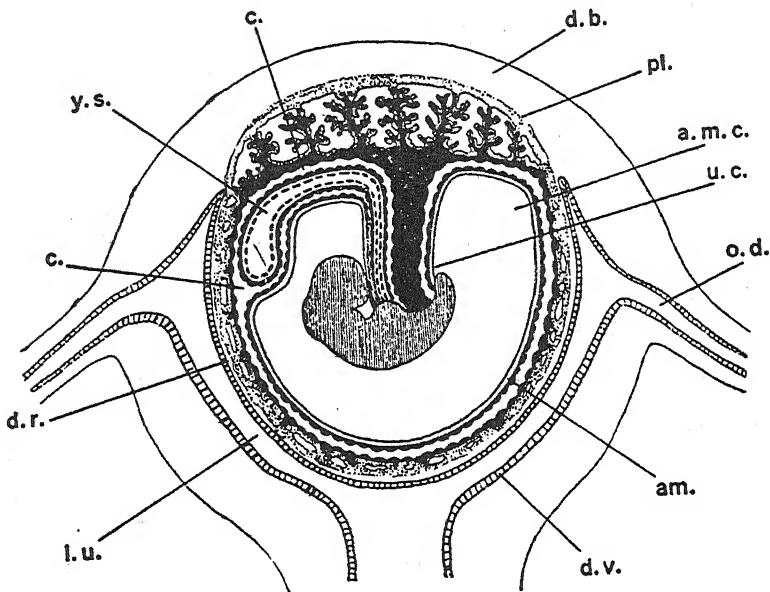


Fig. 288.—Diagrammatic section through the pregnant human uterus and embryo at the seventh or eighth week. From Jenkinson (*Vertebrate Embryology*). After Balfour, after Longet.

am. Amnion. *a.m.c.* Amniotic cavity. The latter has enlarged until it occupies nearly all of the extra-embryonic coelom (*c.*), the amnion being reflected over the umbilical cord (*u.c.*) and yolk-sac (*y.s.*). The yolk-sac, it will be noted, is very small. *d.b.* Decidua basalis (serotina), in connection with which the trophoderm or chorion, represented everywhere by fine stippling, gives rise to the placenta. Thus the chorion in this region is the chorion frondosum. *d.r.* Decidua capsularis (reflexa), consisting of a thin layer of uterine epithelium and mucosa. It soon disappears, exposing the vacuolated trophoderm (chorion) beneath, which in this region becomes the chorion laeve. *d.v.* Decidua vera, whose epithelium also disappears when the trophoderm beneath the capsularis (chorion laeve) comes in contact with it. *l.u.* Lumen of uterus, presently obliterated. *o.d.* Oviduct whose direction in the non-pregnant uterus would be nearly horizontal. *pl.* Placenta; for details see Fig. 286.

of the placenta, however, that the chorion normally continues to be vascularized and to thicken by the growth of villi.

The placenta, as so far described, consists then essentially of a greatly thickened layer of trophoderm containing lacunae or sinuses filled with maternal blood, while into and across these sinuses extend chorionic processes or "villi" containing fetal connective tissue and capillaries. The layer thus indicated is obviously essentially tissue of embryonic origin, and is sometimes known as the "placenta proper." Between it and the muscular wall of the uterus there still exists a certain amount of

the uterine mucosa, i.e., that part of the mucosa which the trophoderm has not destroyed. It now remains to state that in some of the higher Apes and Man (as well as in certain of the lower animals already discussed, e.g., the Cat) this portion of the mucosa is itself differentiated

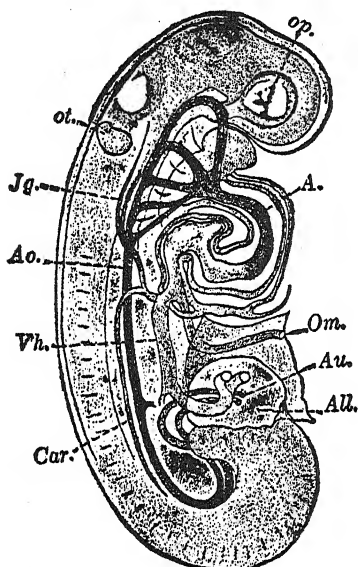


Fig. 289. — Reconstruction of a human embryo of 2.6 mm. From Minot (*Laboratory Text-Book of Embryology*). After His.

A. Aortic limb of heart. All. Body-stalk. Ao. Dorsal aorta. Au. Umbilical arteries. Car. Posterior cardinal vein. Jg. Anterior cardinal vein (internal jugular). Om. Omphalomesenteric vein. op. Optic vesicle. ot. Otocyst. Vh. Right umbilical vein.

into two main layers. The outermost of these layers adjacent to the muscularis is filled with glands, and is known as the *spongiosa*. The second layer, to which the trophoderm is firmly adherent, and in which it is in fact slightly embedded, is occupied by the straighter smaller portions of these glands, i.e., their necks, and is called the *compacta* (Fig. 286). Moreover, the compacta and spongiosa not only exist in the region of the placenta, but likewise at all other points around the uterine wall.¹⁶ Thus, when the non-placental trophoderm of the enlarging blastocyst eventually comes into contact with this wall from which the epithelium soon disappears as indicated in the preceding paragraph, it becomes here also adherent to the compacta. During the later stages of pregnancy, both the compacta and spongiosa tend to degenerate and to become stretched and thin. It is then through the region of either one or both of these layers that the tissue breaks at the time of parturition.

This completes the description of the placenta and the adjacent regions in Man and the Apes. It remains, however, to indicate the names by which the various parts are known in human embryology. To understand the significance of this nomenclature, the student must bear in mind the older idea that placentas of this type were truly deciduate.

¹⁶ The spongiosa and compacta indeed occur not only in the pregnant Primate uterus, but in the non-pregnant uterus as well, particularly just previous to menstruation.

That is, it was thought that a large part of the uterine wall was deciduous, i.e., torn away or shed at parturition. Hence those layers of the wall (i.e., the mucosa) which were supposed so to behave were termed the *decidua*. Also in correlation with this idea, most of the placenta and the covering of the blastocyst was supposed to be formed out of this decidua, rather than out of trophoderm. With this in mind, the reasons for the following names are fairly evident:

That part of the uterine wall to which the placenta is attached is known as the *decidua serotina*, or *decidua basalis* (Fig. 288). The portion of uterine mucosa and epithelium which, during the earlier development, covers the blastocyst on the side opposite the placenta, is called the *decidua reflexa* or *decidua capsularis*. That is, this portion is, as it were, reflected over the blastocyst, forming a cover or capsule for it. Lastly, the remaining part of the uterine wall with which the thin chorion, now lack-

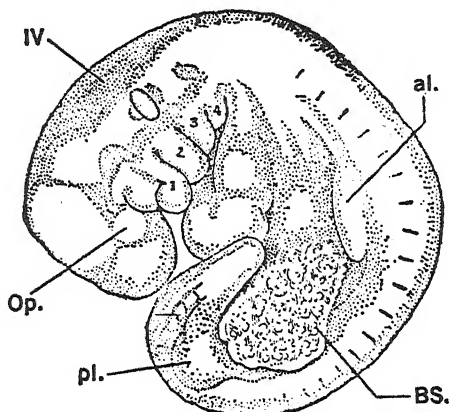


Fig. 290. — Human embryo of about 23 days (4.0 mm.). From Minot (*Laboratory Text-Book of Embryology*). After His (*Embryo a*).

al. Fore-limb bud. *BS.* Body-stalk. *Op.* Optic vesicle. *pl.* Hind-limb bud. *IV.* Fourth ventricle of brain. *1.* Mandibular process. *2.* Hyoid arch. *3, 4.* Third and fourth visceral arches.

ing the overlying decidua reflexa, finally comes in contact, is known as the *decidua vera*, and as this contact occurs the decidua vera disappears down to the compacta. Not only are the parts of the uterus thus named, but the parts of the chorion are also defined. That part which forms the placenta and adheres to the decidua serotina is termed the *chorion frondosum*. The remainder, at least after its loss of the first slightly developed "villi," is the *chorion laeve*.

Comparing the means of embryonic nourishment in the Primates with those in the Rodents, there appears at least one notable difference. In the Rodents the yolk-sac probably plays at least some part in obtaining nutriment for the embryo throughout development; in the Primates (except the Lemurs), on the other hand, this function, as well as that of respiration, is entirely subserved by the placenta. Coming to the actual structure of this organ itself, there exists a striking similarity between

the two orders. There is also, however, a slight difference here, which is perhaps worth noting. At the time of parturition in the Rodents scarcely any maternal tissue, save blood, is lost, and hence the placenta is not at all deciduate in the strict sense of the word. In the Primates, on the other hand, there is a certain amount of the compacta and perhaps of the spongiosa lost at birth, and this is maternal tissue. Hence the Primate placenta, at least to this slight extent, may be said to be truly deciduate. The body-stalk in the two groups is in general similar in lacking any extensive endothelial element. As has been noted, however, its method of formation is different.

DVELOPMENT OF THE PIG TO THE TEN MILLI-METER STAGE

IN the preceding comparative discussion of the early stages of various representative groups of Mammals we have carried the history of the Pig in particular to about the thirteenth day of its development. This means of course thirteen days from the time of fertilization in the upper part of the oviduct. During this time, as we have seen, the egg has reached the uterus, developed into an elongated blastocyst, and the blastocyst is becoming implanted. The embryo itself is represented by a blastoderm in which a primitive groove and notochord are evident, and in which the three primary germ layers have already been differentiated as previously described. The nature of the archenteron, and its relation to the blastocoel has also been indicated.

Having reached this point, we are now prepared to proceed with a description of the further development of this animal. In doing so we are once more faced with the problem of whether to describe the complete development of one system at a time, or to carry all systems along together as it were, in a series of stages. For fairly obvious reasons it is not practical in the case of the Mammal to proceed very far by daily periods. Furthermore, through study of the Frog and Chick we are now familiar enough with the vertebrate plan of development so that we are aware in a general way of what other systems are doing while we concentrate our attention upon one. For these reasons a sort of compromise between the system plan and the stage plan becomes possible. Beginning at the present point therefore we shall carry each system of the Pig to completion in two main steps. The first step will take us to the condition which exists at the 10 mm. stage (20-21 days), a condition more or less comparable with that of a 4-5 day Chick. The second step will then bring the system in question to completion, or as near to it as it is necessary to go. As we proceed with these steps, however, it is desirable from time to time to mention the number of somites present, and also the approximate length of the embryo. In the latter connection certain facts concerning the general form of the animal need to be mentioned,

and we shall take those up at this point, together with a few comments on other external features.

Embryonic Flexions and Rotation. — As in other Vertebrates, so in the Pig, the very early stages pose no question as to what line constitutes the longitudinal embryonic axis. This is obviously indicated by the line of the primitive groove and notochord, and presently also by the line of the fused neural folds, and the contours defined by the folding off of the embryo. This simple condition persists up to about the

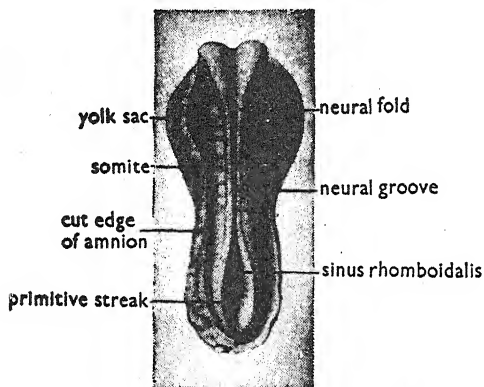


Fig. 291. — Surface view of a Pig embryo of 7 somites (3 mm.), showing closing of neural folds. Amnion removed. After Keibel.

ten somite stage, when the embryo is approximately fifteen days old and measures from 3 to 4.5 mm. in length (Fig. 291). Shortly after this, however, as in the Bird, various curvatures begin to develop, and certain flexures are again recognized. The *cranial* and *cervical flexures* are the same as in the Chick, and in addition two others are named which might also be designated in the Bird, but usually

are not. These are the *dorsal* and *lumbo-sacral flexures* which refer simply to the successively more posterior parts of the continuous curvature. The caudal flexure mentioned in the account of the Chick also exists in the Mammal as a continuation of the lumbo-sacral flexure, but is not generally especially designated (Fig. 294). It should also be noted that for a brief interval before the caudal and lumbo-sacral flexures develop there is, as was also true of the Chick, a slight ventral bend in the mid-body region due again apparently to the pull of the yolk-stalk (Fig. 292). This, however, is quite transitory. As soon as these curvatures develop the question at once arises as to which of the infinite number of straight lines which might be drawn through the embryo is to be designated as its length. In Mammalian embryos, including Man, there are two such lines which are quite commonly used. One is a line passing from the most anterior point of the cranial flexure (mid-brain) posteriorly through the "rump." The latter may be defined as a point at about the middle of the convexity of the lumbo-sacral flexure, i.e.,

somewhat posterior to a point dorsal to the origin of the hind-limbs. This line of measurement is the *crown rump axis*. The other is a line from the posterior side of the cervical flexure, i.e., just over the ear, anteriorly, and again terminating at the rump posteriorly. Because of the position of the anterior point above the ear this may be called the *auricular rump axis*. All measurements referred to in this account will be those of the straight embryo previous to the development of its flexures, and later those of approximately the crown rump axis.

In this general connection one further matter pertaining to the curvatures of Mammalian embryos may be mentioned, though it has no reference to the problem of measurement. It will be recalled that when the Chick developed its various flexures it also acquired a lateral rotation or torsion. In that case this rotation prevented the burying of the anterior end in the yolk. In the Mammal of course there is no

yolk, but it is an interesting fact that the lateral torsion still takes place to some degree (Figs. 292, 293). It is quite variable, as all vestigial structures and activities are apt to be, and soon vanishes entirely.

Other External Features. — Finally before proceeding to a discussion of the specific systems a few further remarks are pertinent with regard to general external features, aside from the various curvatures. As will be apparent from Figure 294, four visceral arches and four "clefts" are in evidence, while about the two posterior clefts is a general depression termed the *cervical sinus*. As sections reveal, however, these are not true clefts since they do not normally actually open through into the corresponding visceral pouches, but it is convenient to refer to them as such. Also from the figure it might at first be supposed

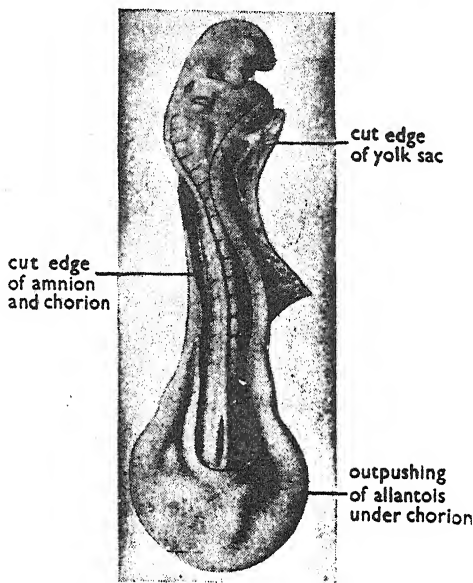


Fig. 292. — Surface view of a Pig embryo with about 16 somites (4.5 mm.), showing outpushing of allantois beneath chorion. After Keibel.

that there are five clefts and five arches rather than four. The apparent first cleft, however, is really the space between the *maxillary process* and *mandibular arch*, and is therefore not counted as a cleft, nor is the maxillary process an arch. Immediately anterior to the maxillary process is still another depression separating this process from the front parts of the face (see below). This depression is the *lachrymal groove*. At its dorsal end is the eye, and at its ventral end the nasal pit. In this connection it may be appropriately noted that one of the few rather striking differences between the appearance of the head of a 4-5 day

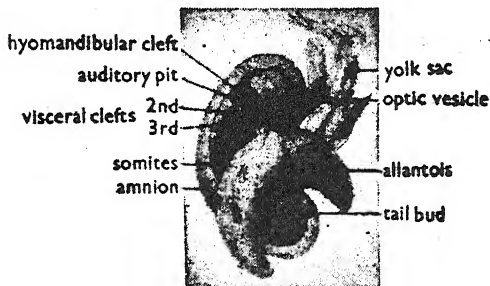


Fig. 293. — Surface view of a 3.5 mm. Pig embryo with chorion removed to show allantois. After Keibel.

Chick and that of a 10 mm. Pig is the much greater size of the eye in the Bird.

Viewing the embryo from the front it will further be seen (Fig. 295) that antero-ventral to the eyes, between them and the olfactory pits, lie the *naso-lateral processes*, which as in the Bird bound the pits laterally.

Medially the pits in the Pig are bounded by the *naso-medial processes*, structures not indicated in the Bird. A comparison of these forms, however, reveals that these last named processes are really only special differentiations (prominences) of the lateral parts of the *naso-frontal process*, which in the Chick is shown bounding the pits on their medial sides. In the Pig the region between the naso-medial processes, i.e., the middle of the "naso-frontal process" is sometimes termed simply the *frontal process*. However, this region is soon (10 mm.) merged with the naso-medial processes which may then be said to join each other in the mid-line. The oral cavity of the Pig soon appears therefore as an opening immediately beneath the fused naso-medial processes. This cavity as usual is bounded ventrally by the mandibular arches, while the maxillary processes are pushing into it from either side. The latter are separated from the naso-lateral processes by the lacrymal groove.

Finally, among external features of the 10 mm. Pig, are the prominent paddle-like fore and hind limb buds and the numerous well-marked somites. Both of course are highly reminiscent of the appearance of these structures in the Chick in a corresponding stage.

THE NERVOUS SYSTEM

As in the case of the Chick, much of the general form of the early mammalian embryo, as well as various prominences appearing upon it, are determined by the developing nervous system. It is therefore convenient to consider this system first.

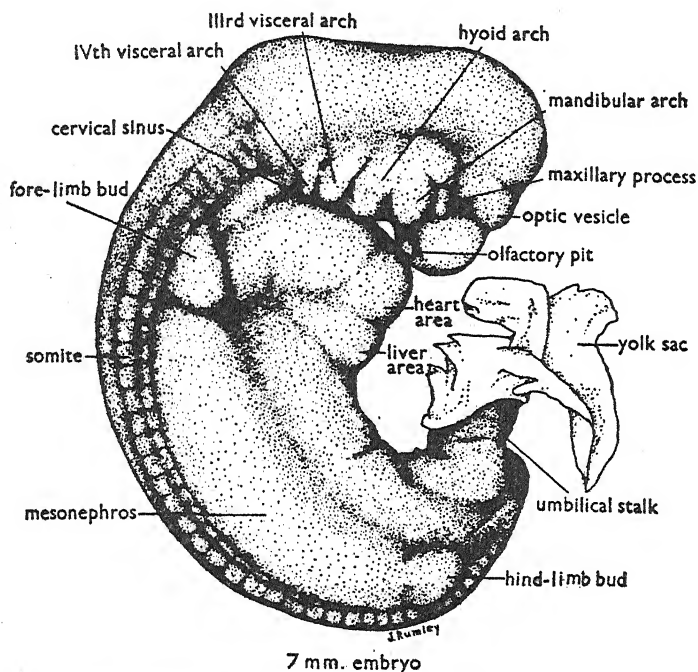


Fig. 294. — Lateral view of a 7 mm. Pig embryo with amnion and chorion removed.

EARLY DIFFERENTIATION

The System as a Whole.—The nervous system first appears in embryos of about 2 mm. as the usual groove in an ectodermal medullary plate immediately anterior to the primitive streak (Fig. 264). Slightly later definite folds arise upon either side of this groove in essentially the same way as in the Bird (Fig. 291). The location where the folds most closely approach each other represents the future hind-brain region, while the wide open part immediately anterior to this is the future fore-brain. The neural tube proper is obviously not yet repre-

sented, which means that the anterior parts of the system are as usual the first to form, and as in other cases maintain their advantage in precocity till very late in development. It will be noted that the chief difference between the situation in the Chick and the Pig at this stage is the wider flare of the folds in the anterior region of the latter. Slightly later,

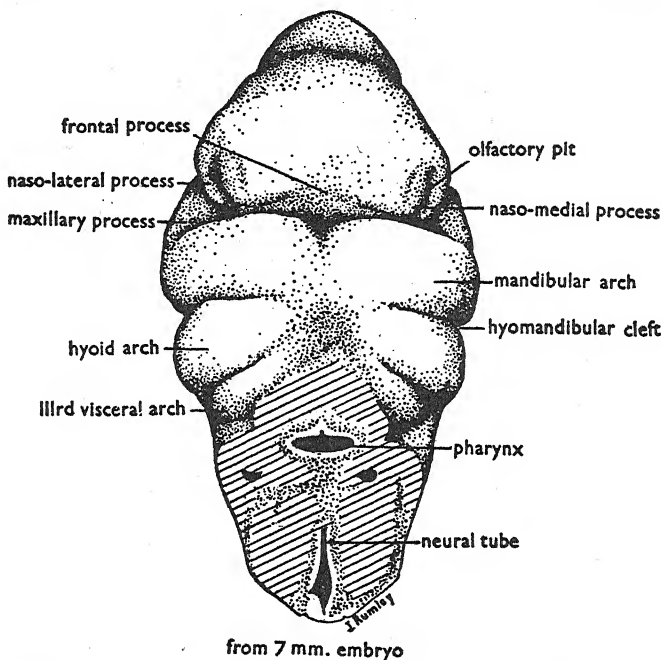


Fig. 295. — Antero-ventral view of the head of a 7 mm. Pig embryo showing parts constituting jaws and face.

at about 10 somites, another difference becomes evident in that, as previously stated, the optic vesicles of the Pig are much less prominent than were those of the Chick at a comparable stage, and this remains true throughout the earlier periods of development. As will be apparent from the figures, these vesicles, at their earlier stages, are also somewhat differently shaped from those of the Bird.

DIFFERENTIATION TO TEN MILLIMETERS

The Brain. — Following this early condition the cranial flexure makes its appearance (13 somites), and shortly thereafter the cervical and caudal flexures are also under way. Thus by the 25 somite stage the anterior extremity is almost touching the heart in about the manner of

a 48-hour Chick with the mid-brain at approximately the most anterior point of the embryo. By this time also the various divisions of the brain are evident, and are the same as those in the Bird, i.e., the *prosencephalon*, *mesencephalon* and *rhombencephalon*. As will presently be noted these main parts are soon further subdivided, and give rise to the same structures as enumerated in the previous form. Thus at 10 mm. (Figs. 296, 297) about the same degree of development of the brain exists, with the same parts in evidence as in a 4-5 day Chick. The prosencephalon is divided into telencephalon and diencephalon, and the former is giving rise to outgrowths (telencephalic vesicles) which will become the cerebral hemispheres. The diencephalon, which is separated from the telencephalon by the same features as characterized the Bird, has, as before, given rise to the *optic vesicles* and the *infundibulum*. The chief difference between this part of the Pig brain at this time, and that of the 4-5 day Chick, is the lack of an epiphysis in the Pig, in which it does not appear until considerably later. The mesencephalon is as usual a very prominent region whose protruding anterior side marks the apex of the cranial flexure. It is, however, not so well developed as that of the Chick at a corresponding stage. This is correlated with the fact that this region is the site of the future optic lobes of the Bird, which are more prominently developed than the partially comparable corpora quadrigemina of the Mammal. A sharp fold, the *isthmus*, separates the mesencephalon from the following *rhombencephalon*, and the division of the latter into *metencephalon* and *myelencephalon* is now distinguishable by the thickened sloping roof which characterizes the former (Fig. 297).

The Neural Tube and Crests. — Passing posteriorly we find that, as in the Frog and Chick, the neural tube has been formed by the closing neural folds so that its dorsal and ventral walls are thin and its lateral walls relatively thick. By the 10 mm. stage the cells in these walls are becoming differentiated into several different types, some of which have already been mentioned in the case of the Chick. Near the delicate *internal limiting membrane* lining the neural canal the original *germinal cells* have given rise to *spongioblasts* and the latter to supporting cells with long fibers running toward the outer periphery of the cord. Again as in the Bird these supporting elements are called *ependymal cells*. The larger part of the cord, however, is occupied at 10 mm. by the *mantle layer*, consisting of other germinal cells in process of further division and differentiation as follows: Some of the germinal cells become spongioblasts which in this layer eventually form other types of supporting cells known as *short* and *long-rayed astrocytes*. The remain-

der of the germinal cells in the mantle layer are *neuroblasts* which later differentiate into actual nerve cells. Finally outside the ependymal and mantle layers, beneath a thin *outer limiting membrane*, there occurs a non-nucleated region termed the *marginal layer*. Because of the lack of

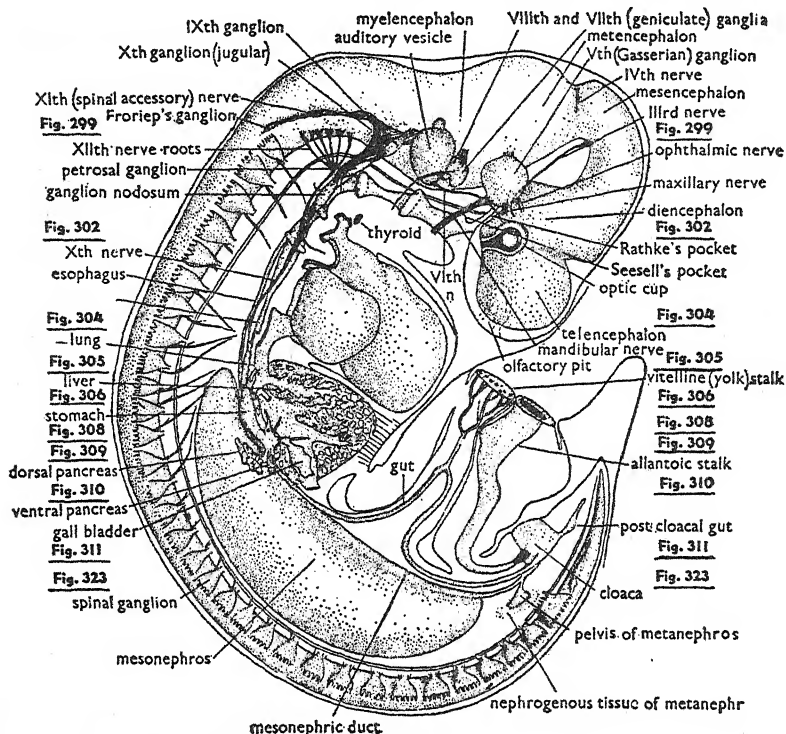


Fig. 296. — Reconstruction of a 10 mm. Pig embryo, designed to show primarily the main features of the nervous, digestive, respiratory and excretory systems at this stage. Drawing made chiefly from a study of sections, with aid from a wax reconstruction produced under the author's direction in the Oberlin College Zoological Laboratory. Lines at the sides with figure numbers over them indicate where the sections represented in these figures pass through the embryo. By laying a ruler along any pair of lines the structures cut by the respective section may be seen.

nuclei, it stains very lightly compared to the darker more central regions. It will further be noted in sections of the 10 mm. Pig that portions of the mantle layer extend ventro-laterally somewhat, causing the lower sides of the cord to bulge slightly. These extensions are the beginnings of the ventral horns (Fig. 298).

Aside from the cord itself it will be found, as in the case of the Frog

and Chick, that as the neural folds come together a band of cells is pinched off between the tube and the overlying ectoderm. The cells of this band soon become concentrated on either side to form the continuous neural crests. The latter are then further concentrated segmentally

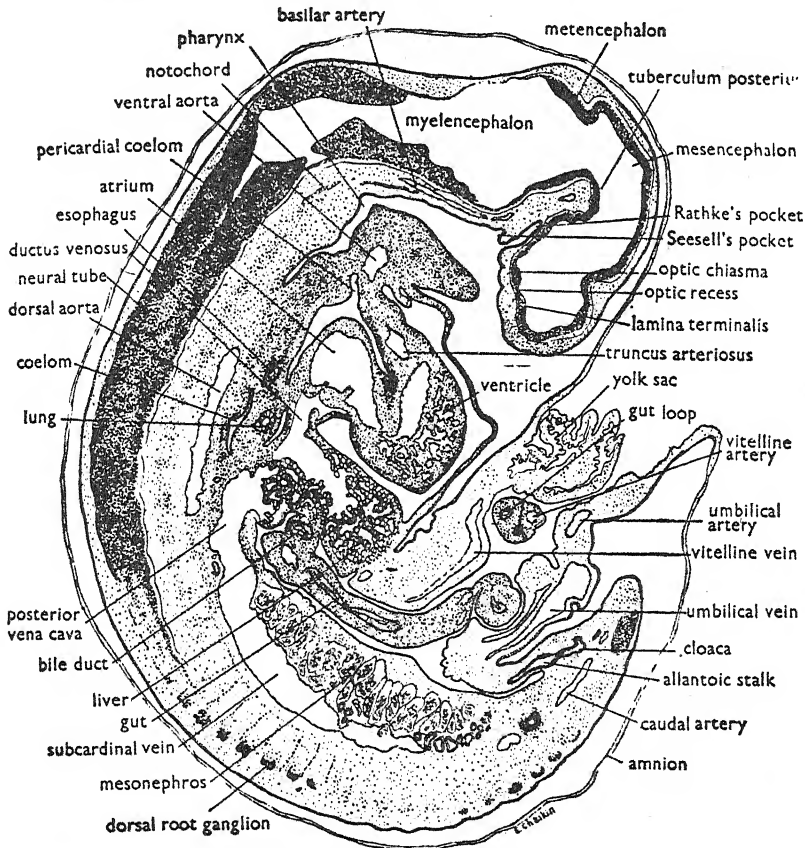


Fig. 297. — Mid-sagittal section of a 10 mm. Pig embryo.

to form the groups of neuroblasts which develop into the spinal ganglia. By the 10 mm. stage each such ganglion is clearly defined, and has given rise to the dorsal roots of the spinal nerves which are definitely connected with the cord.

The Cranial Nerves. — In the 10 mm. Pig all the cranial ganglia and nerves are represented except the I or *olfactory*, and the II or *optic*, the optic stalk not yet containing any actual nerve fibers (Fig. 296).

The III or *oculomotor nerves* can be plainly seen emerging from the ventral side of the mesencephalon, while the IV or *trochelar nerves* are just starting from the dorsal side of the fissure (isthmus) between mid- and hind-brain. The V or *trigeminal nerve ganglion* of each side appears on the ventro-lateral side of the myelencephalon near its anterior end. It is united to the brain by a large root, and from it emerges anteriorly the *ophthalmic nerve*, while more posteriorly and ventrally arise

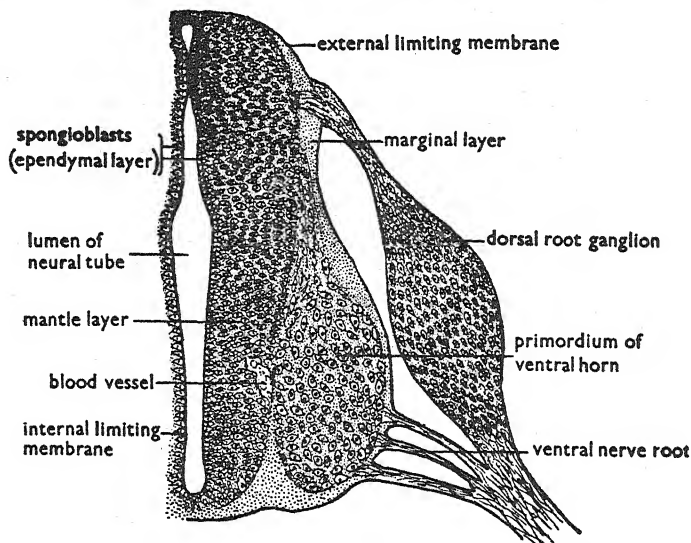


Fig. 298. — Transverse section of the center and right side of the nerve cord and a spinal ganglion of a 10 mm. Pig embryo.

the *maxillary* and *mandibular* nerves. The entire complex lacks the distinct V shape which it had in the Chick due to the large mass of the ganglion proper which obscures the base of the V. More ventral than the V nerve ganglion, at about the middle of the myelencephalon the VI or *abducens nerve* of either side takes its origin, while above it at about the level of the V ganglion occur the ganglia of the VII and VIII nerves. These latter ganglia are somewhat dorso-ventrally elongated structures much less massive than the V. The VII or *geniculate ganglion* is very close to the VIII or *acoustic*, but is slightly anterior to it, and the branches of the VII or *facial nerve* are little developed at this time. The *acoustic* or *auditory ganglion* in turn is in contact with the auditory vesicle which lies posterior to it, the short branches of the *auditory nerve* not being in evidence as yet. There is no single glossopharyngeal

ganglion in the Fig. Instead the nerve cells which would constitute this ganglion are divided into two groups, a dorsal and a ventral. The dorsal group is in close contact with the posterior side of the auditory vesicle, and is called the *superior ganglion* of the IX or *glossopharyngeal nerve*. The ventral group occurs both ventral and slightly posterior to the superior ganglion, and is known as the *petrosal ganglion* of the same nerve. As in the Chick, the X or *vagus ganglion* occurring just behind the IX is also divided into two parts, the *ganglion jugulare* and the *ganglion*

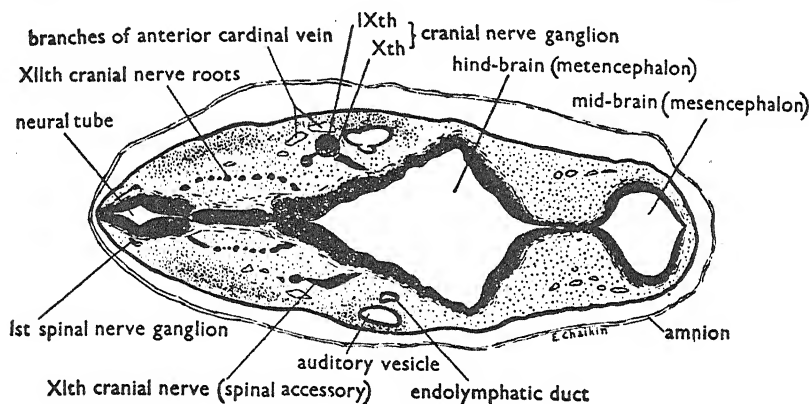


Fig. 299. — Transverse section through the brain region, including some of the spinal ganglia, of a 10 mm. Pig embryo. See reconstruction Fig. 296.

nodosum. The former is so closely in contact with the superior ganglion of the IX at this time as to be scarcely distinguishable as a separate ganglion (Fig. 299). From it there arise two thick strands of nerve fibers. The more dorsal of these proceeds posteriorly to meet the XI nerve, along whose posterior part it extends for a way, as the elongated *commissural* or *accessory ganglion*. The second strand passes postero-ventrally, and shortly enlarges to form the ganglion *nodosum* indicated above. From the latter the *vagus nerve* containing both afferent and efferent fibers is evident at this stage proceeding toward the viscera. The fibers of the XI or *spinal accessory nerve*, already referred to, also pass antero-dorsally from the *nodosum* toward the ganglion *jugulare* along with those of the X nerve. Before reaching this ganglion, however, these fibers branch off in a well-defined strand which curves dorsad, and proceeds along the side of the myelencephalon until it ends in *Froriep's ganglion*. This latter ganglion later disappears, and the nerve is entirely motor. The XII or *hypoglossal nerve* is also entirely motor, and

hence has no ganglion. It arises as a group of fibers ventral to the spinal accessory, and these shortly unite to form a single trunk (Fig. 296).

The Spinal Nerves. — We have already noted the origin of the dorsal root ganglia and the fibers connecting them with the dorsal part of the spinal cord. These are of course sensory nerves. The ventral root motor nerve fibers originate in the ventro-lateral portions of the mantle layer of the cord, whence they emerge opposite each dorsal root (Fig. 298). As in the Chick, they then very shortly join the sensory fibers running outward from the dorsal root ganglion, and from near the point of union three branches arise. The most dorsal branch of each spinal nerve is a *dorsal somatic ramus*, and the middle one a *ventral somatic ramus*, both containing mixed sensory and motor fibers just as they did in the Bird. The third and most ventral branch, also as in the Bird, is a *ramus communicans* of the *sympathetic system*, except in the sacral region whose communicating rami belong to a part of the *parasympathetic system*. The cell bodies which give rise to the fibers of all these rami lie, as in previous cases, within the nerve cord, and are known as *preganglionic neurones*. On the other hand the neurones (*postganglionic*) which constitute the chain ganglia of the sympathetic and parasympathetic systems to which the fibers of the rami run, have as usual migrated thence from the nerve cord, the dorsal root ganglia, or both. This is also of course true of the neurones in the various visceral plexuses. In the case of the Pig, however, it has not been possible to analyze the exact sources of these postganglionic and visceral neurones as carefully as in the Frog and Bird. This is because of obvious limitations on experimental procedure. Also there seems to be no data as to whether the permanent system is preceded by a temporary primary one as in the Chick. Lastly, in connection with the parasympathetic system referred to above, it may be noted that the preganglionic neurones of this system not located in the sacral region, occur in the brain. The parasympathetic and sympathetic systems together are often referred to as the *autonomic system*.

One interesting point concerning the spinal nerves which is true of all the vertebrate embryos with appendages, comes out especially clearly in the 10 mm. Pig. This is the modification in the original strictly segmental arrangement of the spinal nerves. Though this arrangement is still marked, the fusing of several branches in their respective regions to form the brachial and sacral plexuses is very evident. Also the caudal migration of the appendages is indicated by the fact that the branches which form the respective plexuses arise from regions of the cord considerably anterior to the limbs which they supply. The caudal movement

of the diaphragm is likewise evidenced by the anterior origin and backward extension of the phrenic nerve at this stage. In later stages this nerve continues to follow the diaphragm as it moves posteriorly.

The Organs of Special Sense. — As in the case of the parts of the nervous system just described, the organs of special sense in the 10 mm. Pig are also developed to about the same extent as those of a 4-5 day Chick. Thus the *olfactory pits* already noted in the account of the exterior, are present opposite the prosencephalon. Further back the *optic vesicles* have formed *cups* in the usual manner, and each cup is occupied by a hollow sphere of cells destined to become the *lens*. As indicated above, these forerunners of the eye are definitely much smaller relatively than they were in the Bird, but they have formed in the same fashion from the same parts. Likewise the *auditory vesicles* have arisen on either side of the hind-brain by invagination from the surface ectoderm in a way already familiar. They are about the same shape as those of a 5-day Chick with the *endolymphatic ducts* extending dorsalward in the usual manner. As in previous cases these parts are in close proximity to the hyomandibular pouch which will form the middle ear and Eustachian tube (Figs. 296, 299, 302).

THE DIGESTIVE SYSTEM

EARLY STAGES

The Primitive Gut and Related Parts. — We have already noted that in the Pig, as in the Chick, the embryo forms from a flat plate of cells by a folding off process. Also by the time this occurs the germ layers have arisen and the mesoderm has been more or less completely split into the somatic and splanchnic sheets. Hence the innermost layers of the folds which form the gut will consist as usual of the splanchnic mesoderm and the endoderm (*splanchnopleure*). As in the Bird, the folding off is accompanied by the outgrowth of the distal rim of the fold, especially anteriorly and posteriorly. Thus the fore-gut and hind-gut are lengthened (Fig. 300). As in the Bird the proximal rim of the fold, on the other hand, either remains stationary or actually draws together somewhat. Insofar as this latter movement involves the splanchnopleure it produces a great relative narrowing of the *yolk-stalk* or *yolk-sac umbilicus* (see Chick, Fig. 190), so that the gut cavity is more and more sharply separated from the remainder of the extra-embryonic portion of the archenteron. The folds of the somatopleure of course follow, thus narrowing also the *somatic umbilicus*, or as it is called in the Mammal, the *body stalk*, or later the *umbilical cord*.

In connection with this process there are, however, certain differences to be noted between the Chick and Pig. In the first place it appears that the folding off is somewhat more nearly simultaneous anteriorly, laterally and posteriorly in the Pig than it was in the Chick, though even in the former the head fold is a little precocious. A second difference is perhaps more striking, and has already been referred to. It is the fact that at a very early stage the mesoderm develops anteriorly as well as lat-

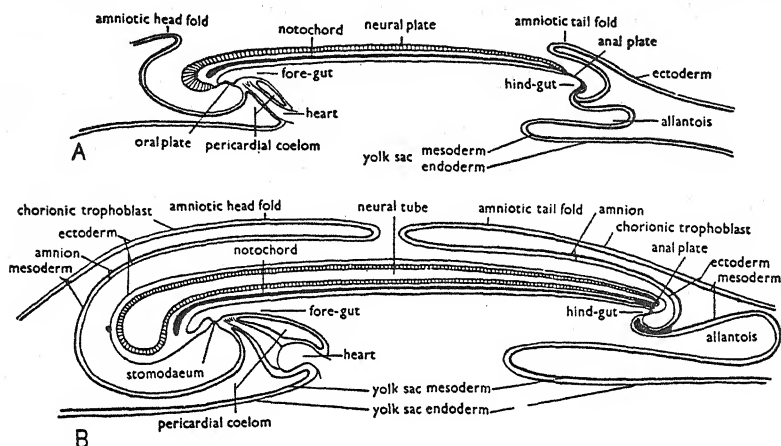


Fig. 300.—Diagrammatic mid-sagittal sections through early Pig embryos to show primarily the method of origin of the allantois which is slightly different from that in the Chick. See Fig. 198. Note also the relatively equal growth of the head and tail amniotic folds as compared with their unequal growth in the Bird.

erally and posteriorly, so that there is no proamnion region which is free of it. Hence the mesoderm is involved in the head fold of the Pig from the first, the same as everywhere else. Still a third difference between Bird and Mammal has to do with the behavior of the mesoderm beneath the forming gut. In both organisms it will be noted that as the lateral folds of the splanchnopleure press toward each other the layers of endoderm are the first to meet. Whereupon they fuse and at once close off to form the completed endodermal tube, save for the opening of the yolk-stalk. The splanchnic mesodermal layers of the splanchnopleure meet next and fuse, but do not close off. Instead they remain as a double sheet, the *ventral mesentery*, which unites the gut to the ventral body wall formed by the subsequent fusion of the somatic mesoderm and ectoderm. In both Bird and Mammal the dorsal part of this mesentery persists to help support the heart and liver. In the Bird, however, the most ventral part, i.e., the part which makes contact with the body wall,

it may be recalled, almost immediately disappears. In the Mammal, on the other hand, this part persists much longer. Indeed in the latter, as we shall see, some of it exists permanently, and we shall have occasion to return to it later on.

The Yolk-Sac. — While the folding of the splanchnopleure is forming the gut and yolk-stalk, what remains ventrally of the original archenteric space becomes the *yolk-sac*. The endodermal lining of this sac

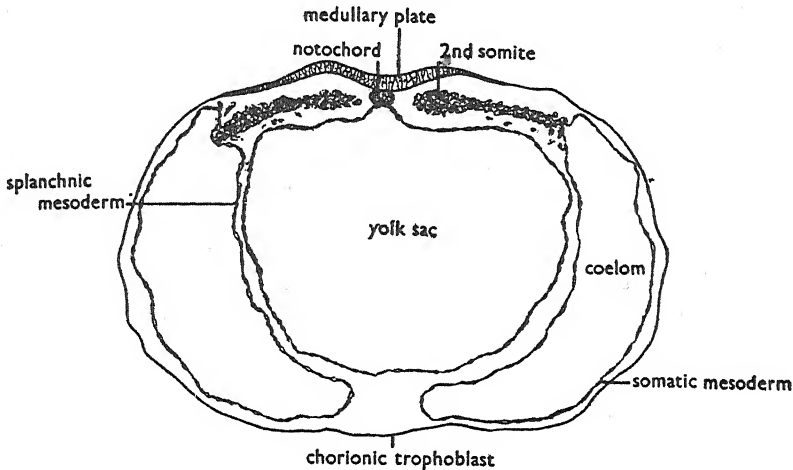


Fig. 301. — Transverse section through a Pig blastocyst cutting the blastoderm and embryo at the level of the second somite. After Streeter, modified to complete the blastocyst ventrally. The embryo is the same as that reconstructed in Fig. 265, and measures 1.56 mm. in length.

has of course been completed ventrally by the growth of this layer clear around the inside of the original blastocoel. The downgrowth of the mesoderm followed by its split into two layers, however, proceeds more slowly. Thus there is a time when this split mesoderm is pushing its way ventrad and medially from both sides, but has not yet met ventrally (Fig. 301). Shortly, however, it does meet, thus everywhere separating the endoderm of the yolk-sac from the trophoblast by a layer of extra-embryonic splanchnic mesoderm, the extra-embryonic coelom and a layer of extra-embryonic somatic mesoderm.

The Allantois. — As the above events are taking place (2–4.5 mm.), it should be noted that at the posterior end of the embryo a condition exists which at first seems very similar to that which prevailed in the Bird. Thus as in that case there is the same fold of the splanchnopleure which in the Bird we have called hind-gut, but which some have

chosen to interpret as allantois. So far as the detailed events in this region have been described for the Pig, however, the subsequent differentiation of the actual allantois and the definitive hind-gut appear to differ somewhat from the history of these parts in the Chick. Thus in the latter the original fold constituting the primordial hind-gut (by some labeled allantois) is, according to our previously stated position, only partly allantoic. This was on the ground that it is not until after the tail-bud has swung around to the ventral side that a portion of this re-

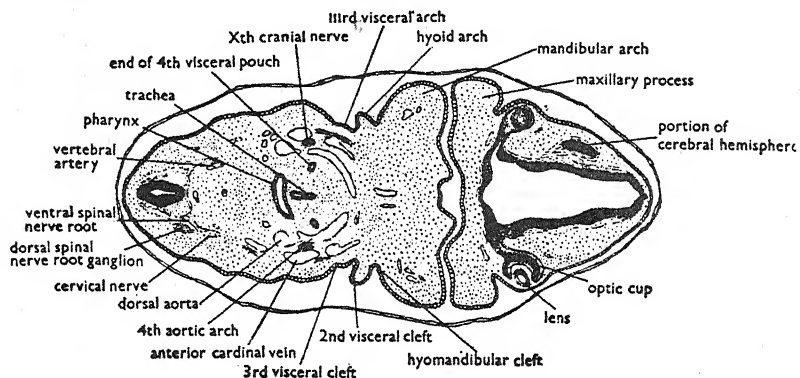


Fig. 302. — Transverse section through the eye and visceral arch region of a 10 mm. Pig. See reconstruction Figs. 296, 318, 320.

gion gives rise to an anterior outgrowth which is entirely allantoic. In the Pig, on the other hand, all of the original posterior fold continues its backward growth to form allantois. Shortly afterward another fold develops in the dorsal splanchnopleure slightly anterior to the allantoic outpushing, and grows posteriorly above the latter to form the definitive *hind-gut* (Fig. 300).

FURTHER DEVELOPMENT OF THE GUT

The Stomodæum. — As in the Chick the fore-gut does not at first open to the outside. Soon, however, the ectoderm becomes invaginated to meet the endoderm at a point slightly posterior to the extreme end of the gut. This invaginated ectoderm is as usual the *stomodæum*, and the double membrane formed by its fusion with the endoderm is the *oral plate*. Sometime between the 15 and 25 somite (4.5–6.5 mm.) stage, this plate breaks through, and puts the stomodæal cavity in communication with the future pharynx. The short portion of gut extending anterior to the stomodæum is a temporary structure known as the *pre-oral gut*, or

in the Mammal as *Seesel's pocket* (Figs. 296, 297). The stomodaeum itself later gives rise to the oral region involving the nasal, maxillary and mandibular processes. At 10 mm., however, the only structure which it has produced is an anterior outgrowth in the direction of the infundibulum of the brain. This diverticulum, as in the Chick, is *Rathke's pocket*,

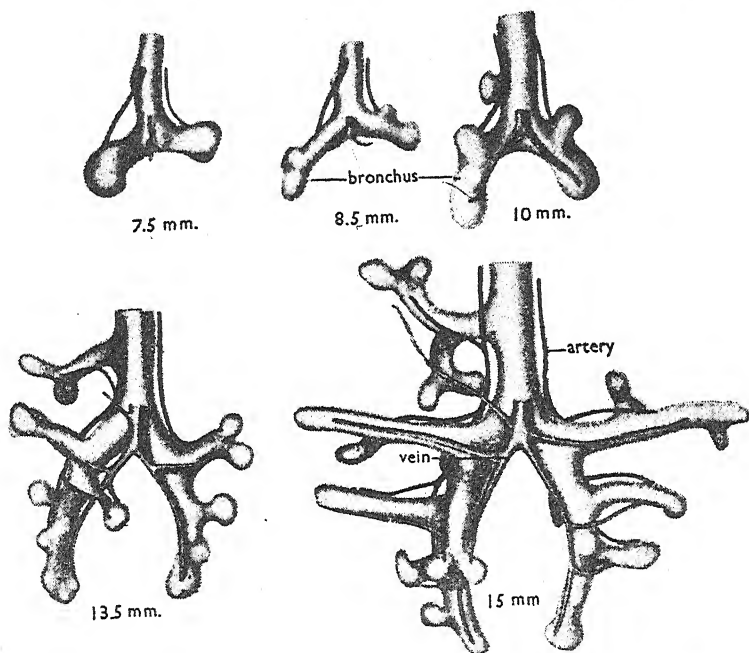


Fig. 303.—Reconstructions of the developing bronchi of a Pig's lung at the stages indicated. After Flint. The arteries and veins, though only labeled in one figure, are represented in the same manner in each.

and is of course, the primordium of the anterior part of the pituitary. (See footnote on this topic in the section on the Frog.)

The Pharynx.—This region of the gut is rather shallow dorso-ventrally, and at an early stage begins to show the lateral outpocketings which form the *visceral pouches*. There are usually four pairs of these in the Pig, the hyomandibular and three posterior to that pair, though the last (fourth) pair are small and sometimes entirely lacking (Fig. 302). In a 10 mm. specimen all the pairs destined to appear are well developed, and have come in contact with the corresponding ectodermal "clefts" (Figs. 294, 296). As already indicated, in the case of the Pig, it is to be noted that, as in most other Mammals, these regions of con-

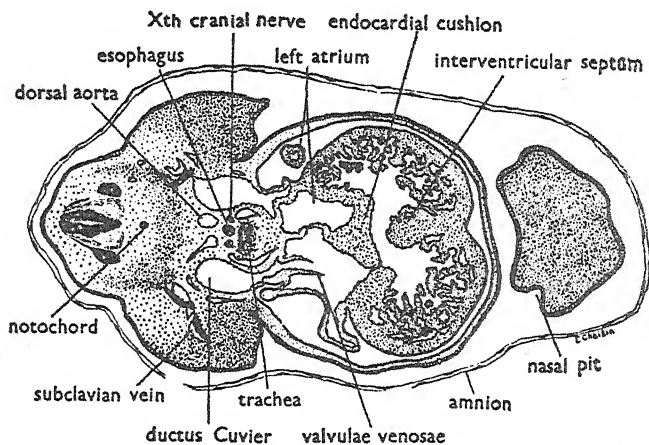


Fig. 304. — Transverse section through the heart and tracheal region of a 10 mm. Pig. See reconstruction Figs. 296, 318, 320.

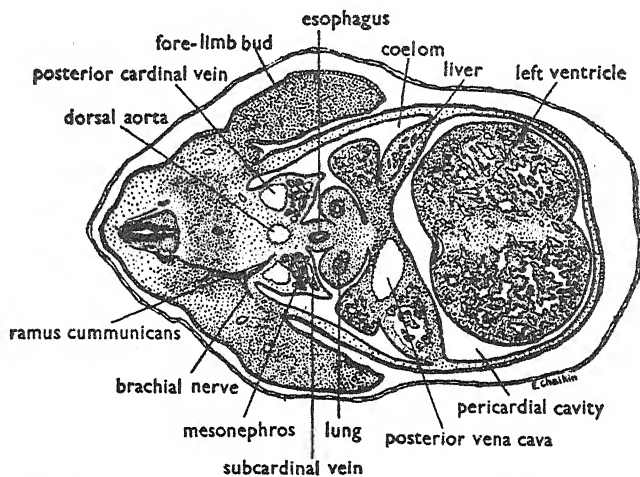


Fig. 305. — Transverse section through posterior of heart and the lung region of a 10 mm. Pig. Umbilical stalk not included in figure. See reconstruction Figs. 296, 318, 320.

tact seldom become perforated, so that no real visceral slits are formed. In occasional instances, however, such perforations do occur even in Man, as reminiscent anomalies, while in the Cow the second pair regularly develop slits for a brief period (Anderson, '22).

The Trachea and Bronchi.—Just posterior to the visceral pouches the pharynx develops a deep ventral groove which, as in the

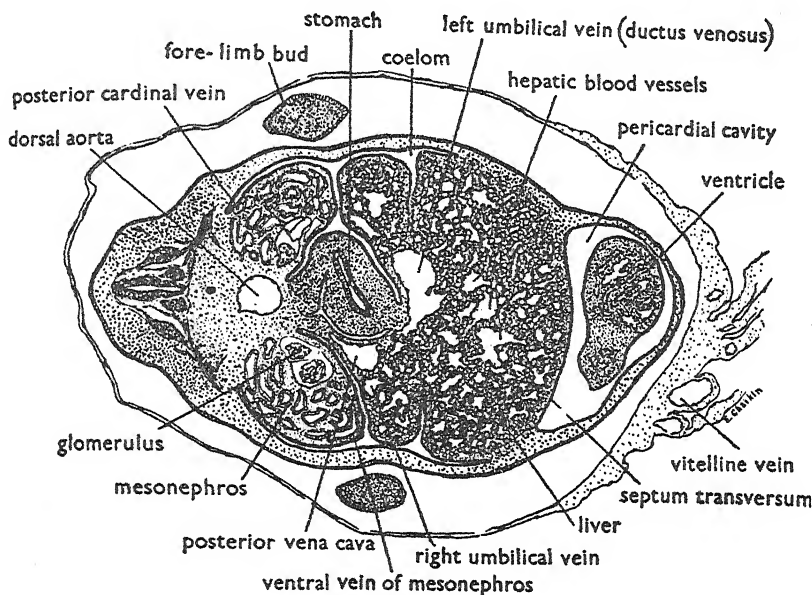


Fig. 306. — Transverse section through the region of the stomach, liver, and posterior tip of heart of a 10 mm. Fig. See reconstruction Figs. 296, 318, 320.

Bird, is the *laryngo-tracheal groove*. As in that case also it shortly becomes converted into a separate tube the *trachea*, which at the 7.5 mm. stage has already produced a couple of outgrowths at its posterior end. These of course are the primordia of the main *bronchi*, though they are commonly referred to as lung buds. At 10 mm. they in turn are just starting to give rise to stubby outpushings, the beginnings of the *bronchial tubes* (Figs. 296, 303, 304, 305).

The Esophagus and Stomach.—Above the trachea the part which remains after the former structure has been pinched off beneath it, is the *esophagus*. Between the 5–10 mm. stages a dilation develops in the enteric tube at the posterior end of the esophagus just behind the limb buds. It is the beginning of the *stomach* (Figs. 296, 306).

The Liver and Related Parts. — In the Pig the *liver* primordium arises as a single rather wide diverticulum from the ventral side of the gut immediately caudal to the stomach region (*duodenum*) at about the 4 mm. stage. In the Bird, it will be recalled, there were two original hepatic outgrowths. The single outgrowth of the Pig, however, very shortly gives rise to several anteriorly directed buds which grow out into numerous hepatic ducts. The posterior part of the same outgrowth becomes extended as the *cystic duct* while its end enlarges as the *gall*

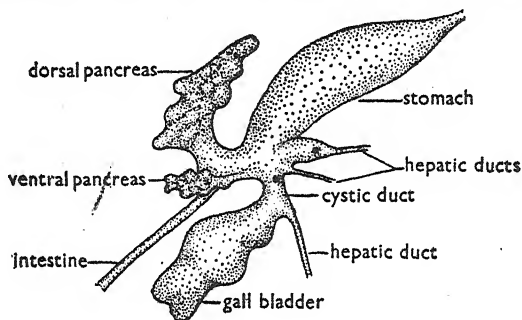


Fig. 307. — Reconstruction of the stomach, dorsal and ventral pancreas and gall bladder of a 10 mm. Pig, enlarged from Fig. 296.

bladder. The anteriorly growing hepatic ducts and the posterior cystic duct remain connected with the gut by the original single outgrowth which becomes extended as the *common bile duct* or *ductus choledochus* (Figs. 296, 307, 308, 309). All these structures, it should be noted, do not just lie freely in the coelom, but are, as in the Chick, embedded within the ventral mesentery whose existence in this region has been previously explained. Their development to the point indicated occurs between the 5–10 mm. stages.

The Pancreas. — At about the same time that the liver diverticulum first appears (4 mm.) a dorsal evagination occurs, in this case within the dorsal mesentery, and slightly posterior to the liver outgrowth. It is the dorsal part of the *pancreas*. At 5 mm. a single ventro-lateral pancreatic rudiment has grown out from the ductus choledochus near the point of union of the latter with the gut. It may be recalled that in the Chick there were two of these ventro-lateral pancreatic primordia from the common bile duct, as well as the single dorsal one. At 10 mm. each single dorsal and ventral pancreatic primordium in the Pig consists of numerous budding cords of cells, and the two parts are almost fusing (Figs. 296, 307, 308, 309).

The Mid-gut Region.—Immediately posterior to the liver and pancreatic diverticula the intestine of the Pig, like that of the Chick, turns ventrad. It proceeds in this direction as far as the origin of the yolk-stalk, and then passes dorsad again to the region of the rectum. By the 10 mm. stage the gut in this region has become a rather small tube, and its ventral bending has become a very clear cut loop whose sides are quite closely approximated. At the most ventral point of this loop,

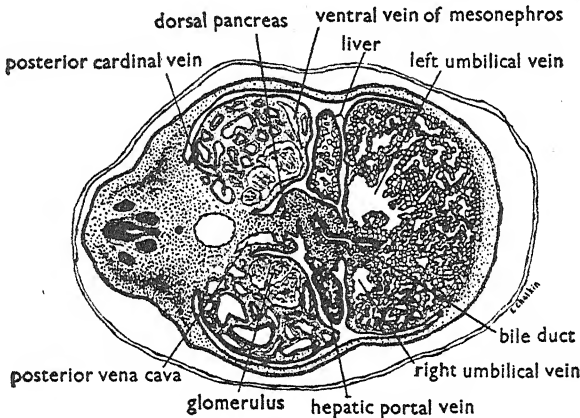


Fig. 308.—Transverse section through the region of the anterior end of the mesonephros, the bile duct and liver of a 10 mm. Pig. Umbilical stalk not included in figure. See reconstruction Figs. 296, 318, 320.

from its rather sharp apex, the yolk-stalk still takes its origin. By this time, however, this stalk is extremely constricted to form an even smaller tube than the intestine, and the yolk-sac at its extremity exists merely as a shriveled vestigial diverticulum within the body-stalk (Figs. 296, 297, 309, 310). In some instances at this time a small enlargement appears on the posterior ascending limb of the loop. It is the beginning of the *caecum*.

The Hind-gut Region.—Continuing posteriorly it has already been noted that an evagination or fold has arisen in the dorsal wall of the splanchnopleure of this region just anterior to the allantoic outgrowth to form the hind-gut (Fig. 300). The crest of this fold is almost from the first in contact with the ectoderm above it, the fusion constituting the *anal plate*. Thus this plate is at first dorsal, just as in the Chick. With the outgrowth of the tail bud the caudal portion of the hind-gut region is drawn posteriorly and ventrad. The result is that the anal

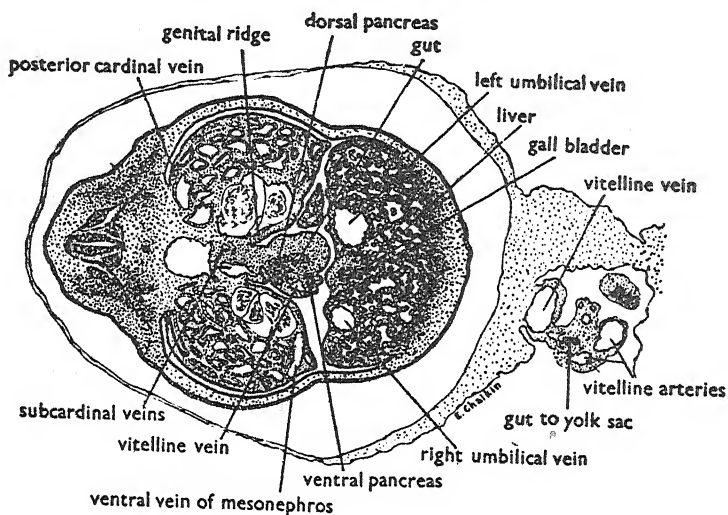


Fig. 309.—Transverse section through the region of mesonephros, pancreas and posterior of liver of a 10 mm. Pig. Only a part of the umbilical stalk included in the figure. See reconstruction Figs. 296, 318, 320.

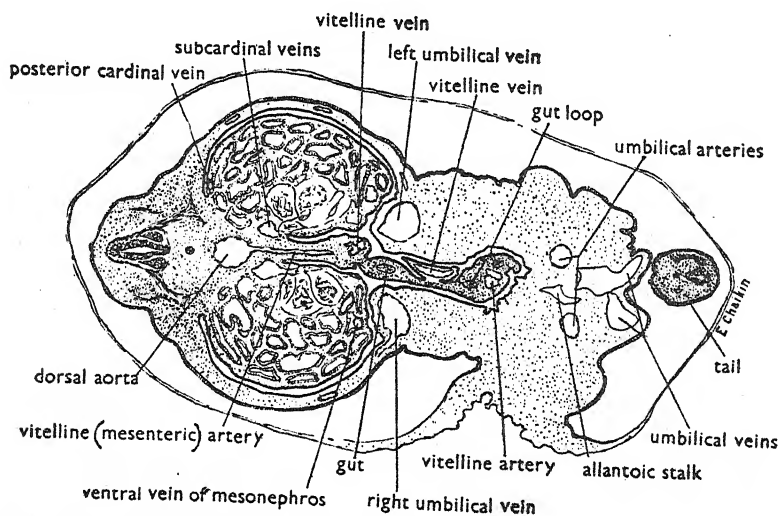


Fig. 310.—Transverse section through the region of mesonephros, gut loop, umbilical and vitelline arteries and veins, allantoic stalk and tip of embryo of a 10 mm. Pig. See reconstruction Figs. 296, 318, 320.

plate, as in the Bird, is presently swung clear around to the ventral side. With the further outgrowth of the tail bud a small portion of the hind-gut is pulled out into this bud a short distance beyond the anal plate. As in the Chick this extension is the *postanal gut*, but unlike the case of the Chick it is entirely a temporary structure with no future function, and so need not be referred to again. Both it and the anal plate, it should be noted, are now caudal and ventral to the allantoic stalk. Thus with the shift in these parts the latter no longer extends pos-

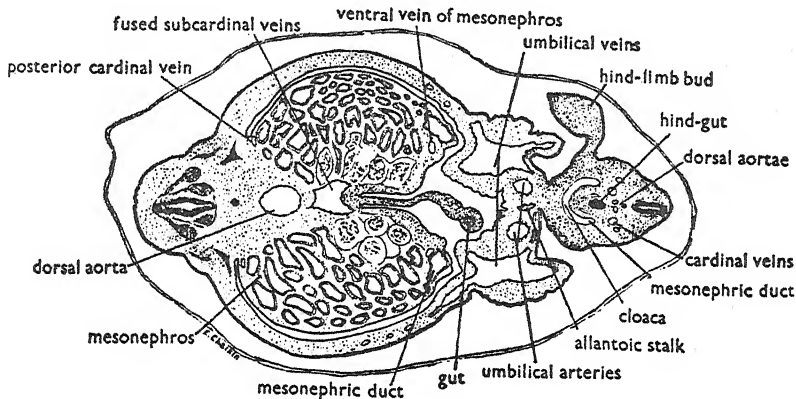


Fig. 311. — Transverse section through the region of mesonephros, gut, umbilical veins, allantoic stalk and cloaca of a 10 mm. Fig. See reconstruction Figs. 296, 318, 320.

teriorly, but rather proceeds at first dorsad before curving antero-ventrally into the body-stalk (Figs. 296, 311). Just within the embryo postero-dorsal to the anal plate, the slightly enlarged end of the gut constitutes the *cloaca*, and the anal plate may now be termed the *cloacal membrane*. This enlarged region of the gut is called the cloaca because as in the Chick it presently receives not only the gut opening (anus), but those of the urinogenital ducts and the allantois. The opening of the anus is furthest postero-dorsal, those of the urinogenital ducts, slightly more cephalad and ventro-lateral, and that of the allantois more antero-ventral (Fig. 296). By the time this situation has developed, e.g., in a 6 mm. embryo, there has also occurred, according to some, the usual depression in the ectoderm surrounding the cloacal membrane to form the *proctodaeum*. The latter, though, seems not to be much in evidence at 10 mm. Thus we have a condition essentially similar to that in forms previously studied. From this point onward, however, the situation in the Mammal begins to diverge from that previously observed.

The divergences just suggested, though not far advanced in the 10 mm. stage, are definitely underway, as a result chiefly of one process. Within the cloaca a crescentic sheet of tissue, the *urorectal fold*, is growing from the postero-dorsal wall toward the cloacal membrane and from the lateral walls toward the median line. When completed the result will be to divide the cloacal chamber into two parts. One, the postero-dorsal into which opens the large intestine, will constitute the *rectum*. The other, antero-ventral, part is called the *urinogenital sinus*, and constitutes essentially an extension of the neck of the allantois which now receives the urinogenital ducts (Figs. 311, 337). Although this change has been initiated in the 10 mm. embryo, the cloacal division is not yet complete, nor is the cloacal membrane yet ruptured as is the case with the oral plate.

MESODERMAL STRUCTURES

Under the headings of systems, we have thus far considered the nervous system, which of course is exclusively ectodermal, and the digestive system. The latter because of its lining is often thought of as primarily endodermal, though of course much of its walls are derived from mesoderm. Now, however, we are about to consider systems which are exclusively mesodermal in origin, e.g., the circulatory system, and the urinogenital system. Before embarking upon our discussion of these definite systems, however, it is also necessary to make a few further comments regarding the condition of the mesoderm in general.

The Somites. — We have already discussed the origin of the lateral plate mesoderm, but there has been no occasion to refer to the somites except in a general way as criteria of development. It may now be noted that these structures develop in the Pig in almost exactly the same manner already made familiar in the Chick. As in that case the first ones formed turn out to be the most anterior, each new one being added between the most anterior old one and Hensen's knot. Not only is the order of their origin similar but their character and method of development is the same. Thus the original ridges of mesoderm adjacent to the notochord and nerve cord first become segmented. Then each segment (somite) becomes a roundish mass with the cells radiating from its slightly hollow center. Next the cells adjacent to the notochord and nerve cord become loosely arranged about these structures as *sclerotome*. At the same time the cells of the dorsal part of the remaining outer wall grow ventrad between this wall and the sclerotome. Thus is formed a new dorso-ventrally elongated double layered structure with

a space between the layers. The outer layer as before is called *dermatome*, and the inner wall *myotome*, the space between them being *myocoel*. The question of what these layers eventually give rise to, is still uncertain in the case of the Mammal as it was in the Bird. The inner layer certainly goes largely to form skeletal muscle, but to what extent the outer layer or dermatome really forms dermis is not so clear. Probably only part of it so behaves. The sclerotome, however, again unequivocally gives rise to the parts of the vertebrae. By the 10 mm. stage the parts of the original somites indicated above are no longer evident, except to a slight extent toward the posterior (Fig. 310).

The Intermediate Mesoderm. — Though this term was not used in the case of the Frog and Chick its equivalent was present. It is merely the mesoderm between the somites and each lateral plate, i.e., it is the part previously designated as *nephrotome*. The latter term indicated its fate in the previous cases, and it is the same here. The details of this will of course be taken up in connection with the urinogenital system.

The Somatic and Splanchnic Mesoderm. — The origin of the somatic and splanchnic mesoderm, has already been discussed, and need not be gone into here. However, it is pertinent to note that by the 10 mm. stage the intermediate mesoderm on each side no longer connects the lateral sheet of that side with the disappearing somites, but throughout much of its length forms a discrete mass, the developing mesonephros (Figs. 305, 309). As the latter pushes out into the coelom it of course carries a layer of mesoderm before it as its covering of coelomic epithelium. It thus comes about that on the median side of each mesonephros this covering passes dorso-medially until the two sheets of epithelium are separated only by the mesentery of the gut. With this arrangement the division between somatic and splanchnic mesoderm might now seem to be somewhat confused. It is customary, however, to designate only the mesodermal covering of the outer body wall as somatic. The remainder covering the mesonephros (and later the metanephros), the mesentery and the viscera is then splanchnic.

THE CIRCULATORY SYSTEM

The Blood Islands. — It will be recalled that in the Bird one of the first manifestations of the beginning of the circulatory system is the formation of blood islands in the area vasculosa, which is of course extra-embryonic. Virtually the same situation obtains in the Pig where the blood islands also appear on the surface of the empty yolk-sac corresponding to the area vasculosa of the Chick. It will be recalled that

in the Bird, however, the mesoderm from which they arise in this region is supposed to have migrated out from the area pellucida. It then forms blood islands, and these in turn bud off mesoderm cells between them and the ectoderm. No such indirect method seems to occur in the Pig. The mesoderm is already in this area, and is divided into somatic and splanchnic layers. The blood islands are then organized out of cells from the splanchnic layer between it and the endoderm. As before, these cells become aggregated into clumps, and while those around the periphery of each clump become flattened to form blood vessel *endothelium*, the more central ones 'transform into *blood corpuscles*. It should be noted also that in the Mammal this activity is not confined to the mesoderm of the yolk-sac. The allantois, which is somewhat more precociously developed than in the Bird, likewise produces blood islands in a similar manner. It has recently been demonstrated, moreover, that in certain Monkeys red blood corpuscles continue to be formed from the endothelial walls of the blood sinuses of the chorionic villi during early pregnancy (Wislocki, '43). It is further claimed that in the Baboon even the amnion produces red blood cells (Noback, '46). While early genesis of blood cells occurs in these various extra-embryonic locations their later formation is relegated to special organs such as the mesonephros, liver, spleen and finally the bone marrow. Meanwhile the differentiation of the endothelium of numerous vessels goes on constantly throughout the embryo. As the circulatory system thus develops it is quickly supplied with both corpuscles and fluid from the various blood islands, and later from the other sources just indicated. Whether these later centers possess their capacity as a result of the migration to them of blood forming mother cells from the original blood islands is still an open question. Some hold this view, while others maintain that the later centers give rise to their own blood-forming cells from local mesoderm. Possibly both methods occur. In any event there are of course many kinds of blood cells produced from the original mother cells, and their varied differentiations make a complicated subject which we shall not go into.

The Heart. — One of the first parts of the intra-embryonic circulatory system to develop is the heart, and the method of its early formation is virtually identical with what we have already described in the Chick. On either side of the pharyngeal region, before this part has been closed in ventrally, the endothelium of a blood vessel forms between the splanchnic mesoderm and the endoderm in the manner described above. As the closure occurs these two blood tubes fuse beneath the pharynx to

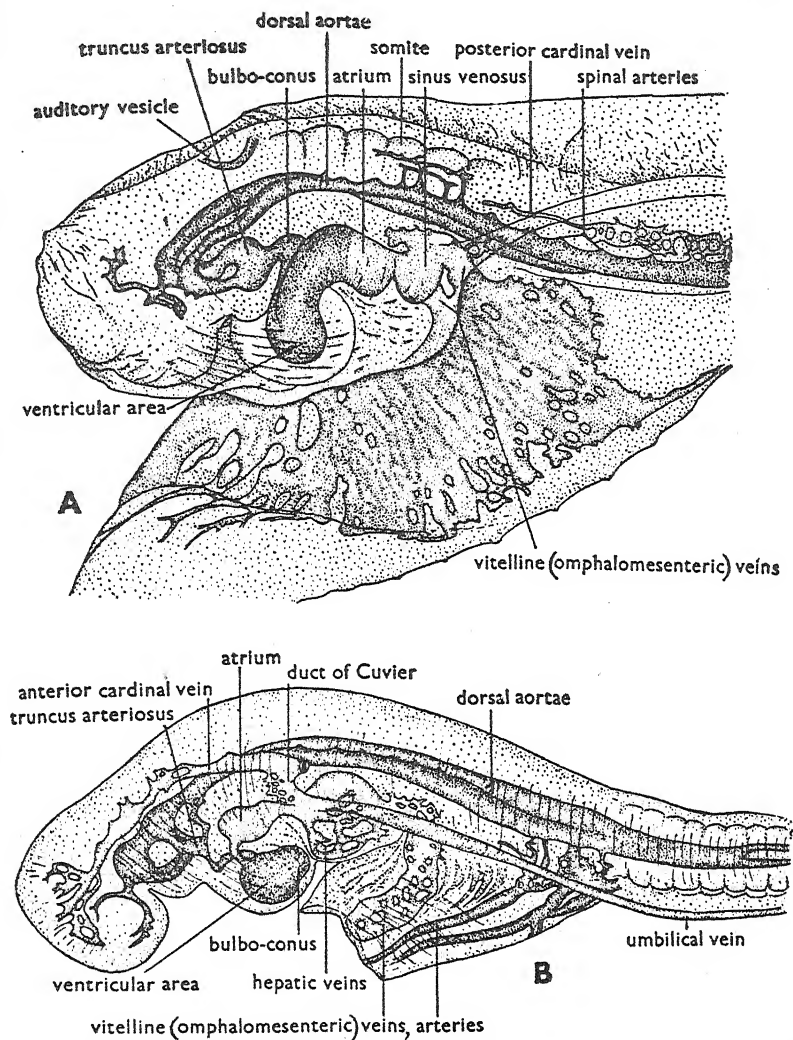


Fig. 312. — *A*. Partial injection of the vessels of a Pig embryo of 14 somites, 4 mm. in length. After Sabin. *B*. Partial injection of the vessels of a Pig embryo of 27 somites, 6 mm. in length. After Sabin.

form the usual single heart tube. The splanchnic mesoderm follows the endothelium and while the latter constitutes the *endocardium*, the mesoderm covers it to form the *epicardium*, and the *dorsal* and *ventral mesocardia*. Because of the latter the two coelomic spaces on either side (in the Bird called the amnio-cardiac vesicles), as in that case, do not at first communicate. Presently, however, the ventral mesocardium disappears, and the two parts of the pericardial space are united. The dorsal mesocardium, as in the Chick, persists somewhat longer. This condition

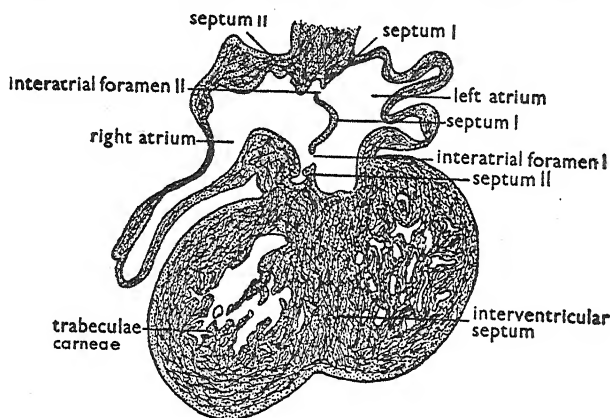


Fig. 313. — Frontal section through the heart of a 10 mm. Pig.

is reached at about the 4.5–5 mm., or 13 somite stage. (See Chick, Fig. 179.)

The next steps in cardiac development in the Pig are again very familiar. The dorsal mesocardium in its middle region disappears, leaving the double-walled tube free to bend. Then as the latter increases in length it becomes thrown into the usual curve to the right, and this shortly becomes a loop whose apex is rotated backward. As in the Chick, the postero-dorsal part of the loop becomes the atrium, the apex of the loop and a portion of each limb the ventricle, and the antero-dorsal end of the more anterior limb the *truncus arteriosus*. These parts then rotate so that the atrial region becomes antero-dorsal, and the apex of the ventricle postero-ventral with the truncus running cephalad along the antero-ventral face of the ventricle. From a comparison of this description and of the figures of the heart of the Frog and Chick at similar stages the essential likeness will be apparent (Figs. 108, 184, 312). By 10 mm. the bendings and shiftings indicated above are complete, and the heart presents externally almost the adult appearance. Inter-

nally a crescentic septum, the *septum primum* (I) has grown from the antero-dorsal wall of the atrium, and has partially divided it into right and left chambers. Postero-ventrally, i.e., toward the ventricle, however, the growth is not quite complete, and the very small opening briefly remaining is all that is left of the originally wide-open orifice between the atria, the *interatrial foramen primum* (Figs. 313, 314). Meanwhile dorso-anteriorly a new opening has developed in the septum called the *interatrial foramen secundum*. Also another septum, the *sep-*

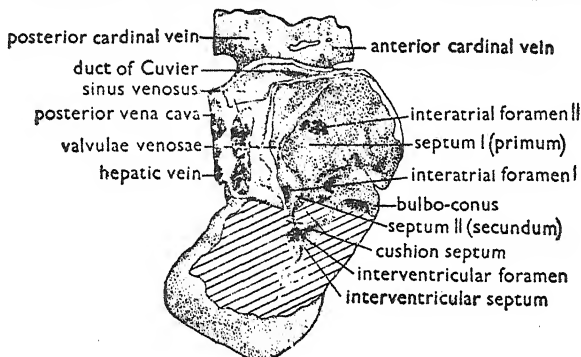


Fig. 314. — Reconstruction of the heart of a 7.9 mm. Pig with the right atrium and right ventricle opened from the right side. After Morrill.

tum secundum (II), is sometimes slightly in evidence to the right of the septum primum (Fig. 313). The further fate of these septa, their openings and their functions will be fully discussed in the section on later development. Another conspicuous structure apparent within the right atrium at 10 mm. is a pair of flaps guarding the orifice from the sinus venosus to this atrium, the *valvulae venosae* (Fig. 304). Later on one of these valves forms a minor ridge, the *septum spurium*, which soon disappears.

Between the atrium and the ventricular region the heart is somewhat constricted to form the *atrio-ventricular canal*, and this also has become almost or quite divided by growths proceeding from its dorsal and ventral walls. When complete these growths, as in the Bird, will form the so-called *cushion septum* (Fig. 304). At the same time a third septum, the *interventricular*, is growing from the apex of the ventricle toward the atrio-ventricular canal (Fig. 304). All these septa will shortly meet to divide the entire organ into completely separated right and left chambers, save for the existence of one of the interauricular foramina which persists until birth and even after. Finally the walls of

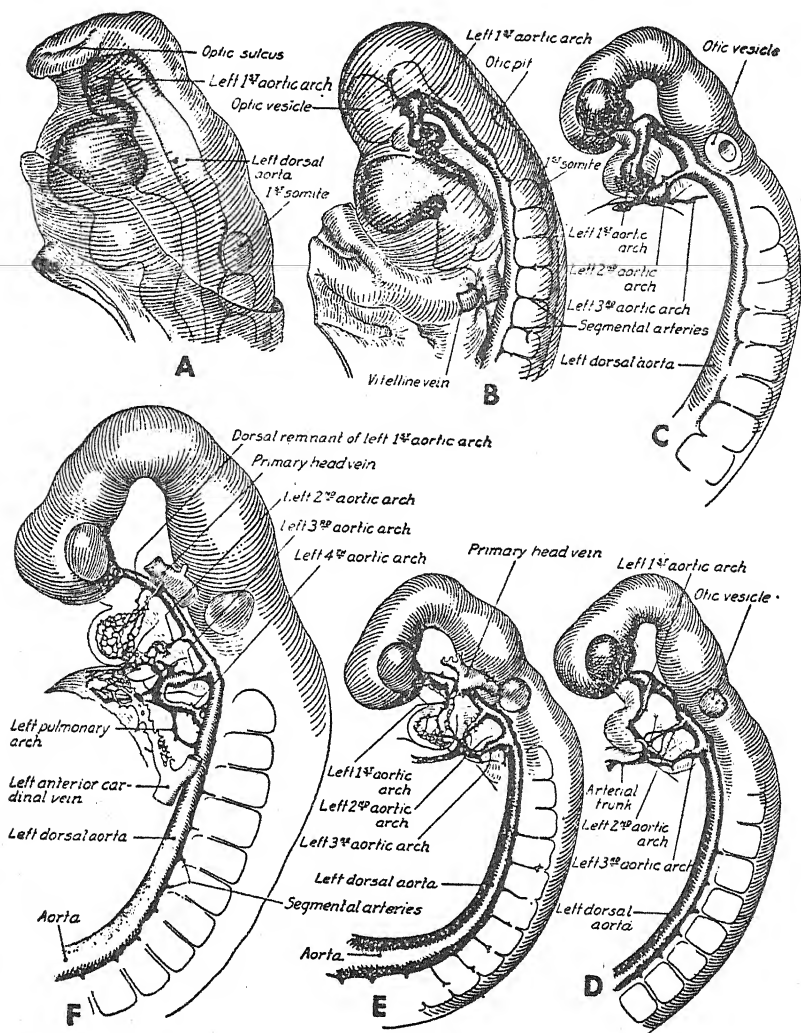


Fig. 315.—Stages in the development of the aortic arches and other anterior arteries in the Pig. After Heuser. A. 4.4 mm., 10 somites. B. 4.15 mm., 19 somites. C. 3.8 mm., 26 somites. D. 4.57 mm., 28 somites. E. 4.46 mm., 30 somites. F. 6 mm., 36 somites. It will be noted in this and other cases that the stages of development as indicated by the number of somites are not always exactly correlated with the relative lengths of the embryos. The former is usually the more accurate criterion of degree of general development in the earlier stages. Hence both items are given.

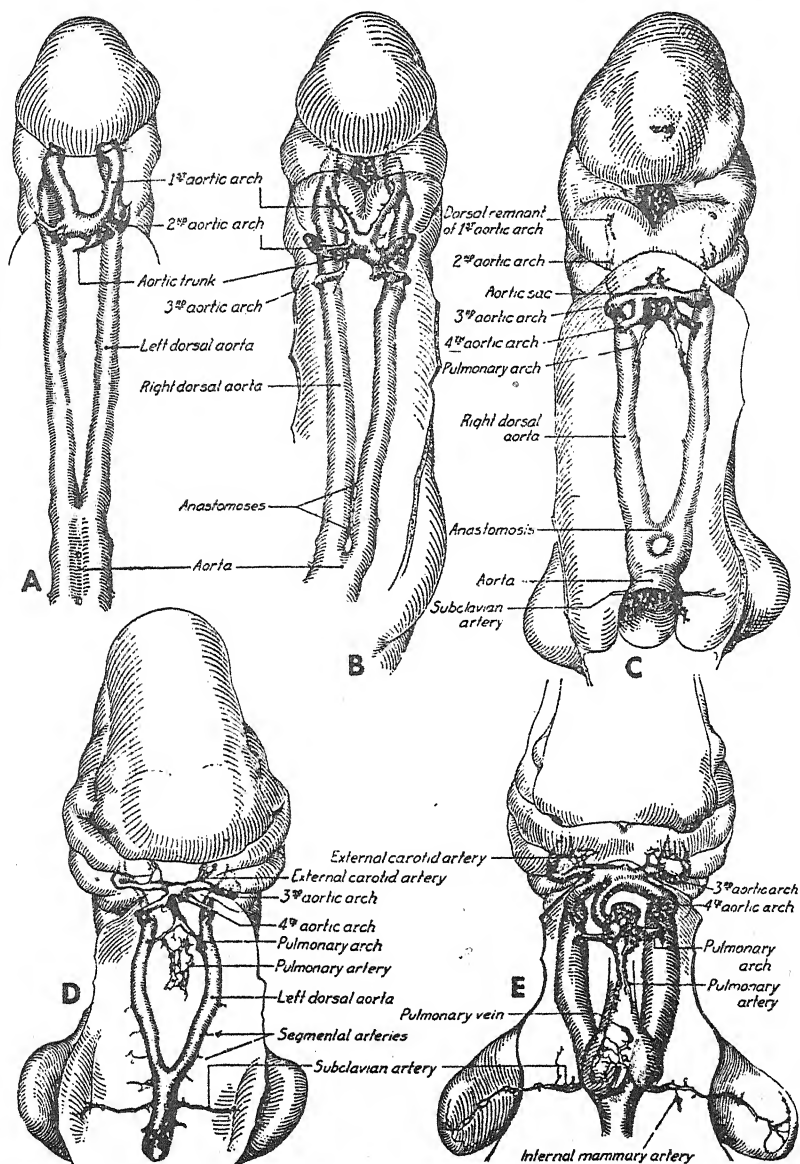


Fig. 316.—Stages in the development of the aortic arches and other anterior arteries of the Pig. After Heuser. A. 24 somites. B. 4.3 mm., 26 somites. C. 6 mm., 36 somites. D. 8 mm. E. 12 mm.

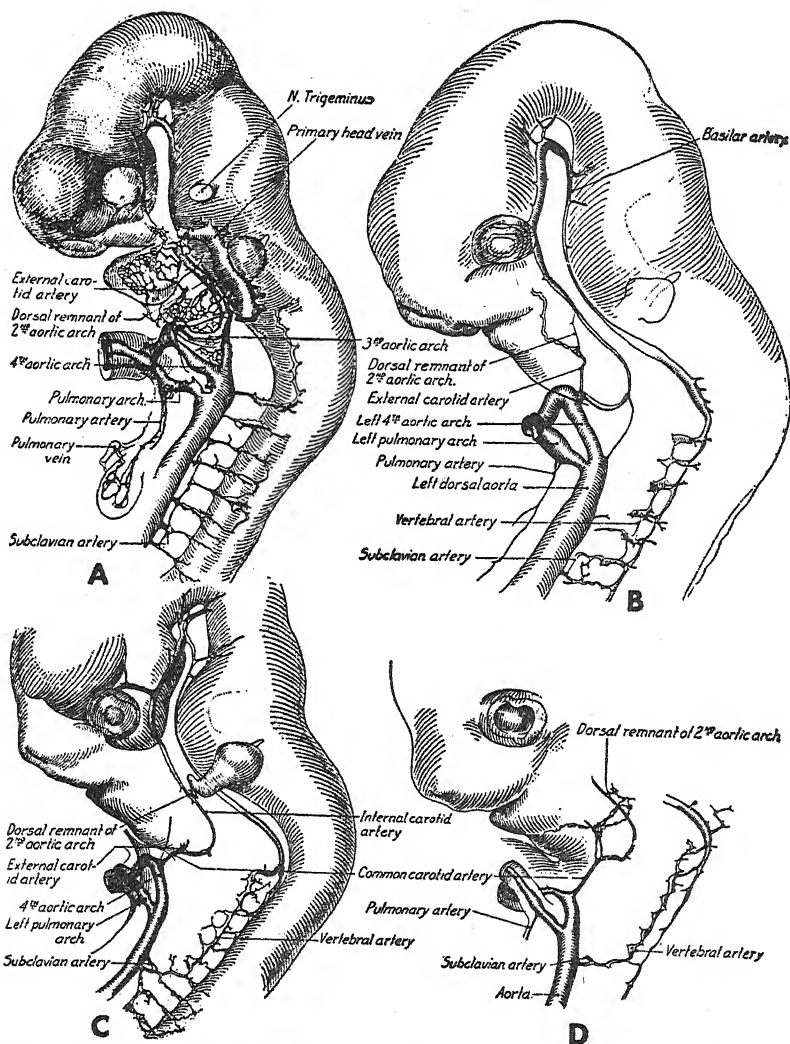


Fig. 317. — Stages in the development of the aortic arches and other anterior arteries in the Pig. After Heuser. A. 12 mm. B. 14 mm. C. 17 mm. D. 19.3 mm.

the ventricles become definitely thickened, and muscular bands, the *trabeculae carneae* project into the ventricular lumen.

The Truncus and Aortic Arches.—The truncus arteriosus has already been mentioned as it comes up underneath the pharynx. As in

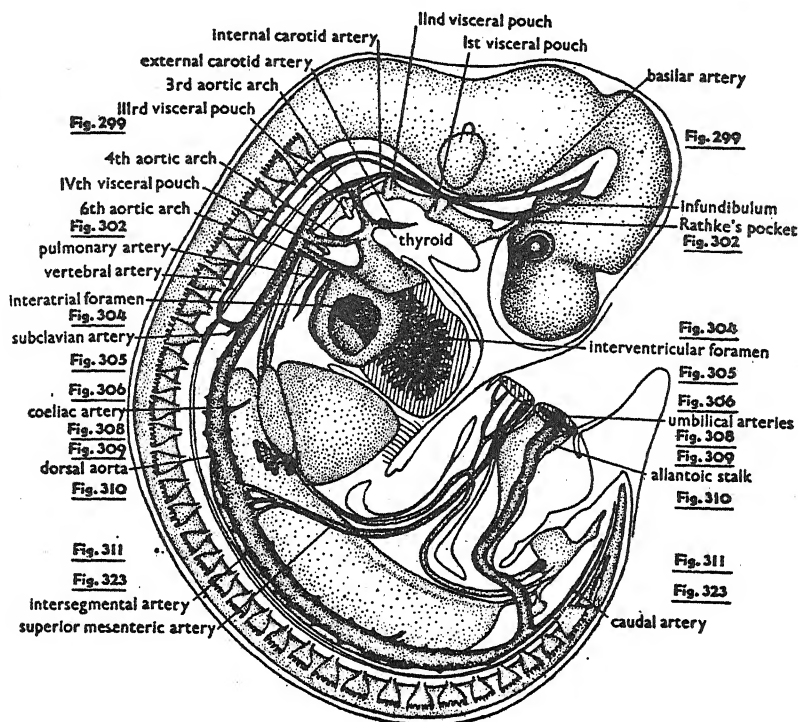


Fig. 318. — Reconstruction of a 10 mm. Pig embryo designed to show primarily the main features of the arterial system at this stage. Drawing made by same methods as used for Fig. 296. As before the lines at the sides indicate where the sections denoted by the figure numbers above the lines, pass through the embryo.

the case of the Chick this large vessel does not, contrary to what most diagrams suggest, really extend any distance cephalad in a horizontal position before giving off the aortic arches. Instead it extends dorsally and only slightly cephalad directly into the midst of the pharyngeal region (Fig. 318). Here it gives rise to the six aortic arches, but again as in the Bird, not all at one time. The mandibular aortic arch appears first, then the hyoid, and by the time the other four pairs have developed in the remaining visceral arches (10 mm.) the first two aortic vessels have disappeared (Figs. 315, 316). Also again as in the Chick, the fifth pair

are vestigial, sometimes appearing briefly as loops on the front sides of the sixth arches, and sometimes on the posterior sides of the fourth. With respect to the sixth arches themselves it must be noted that as early as 7.5 mm. each has given rise to a small posterior outgrowth which

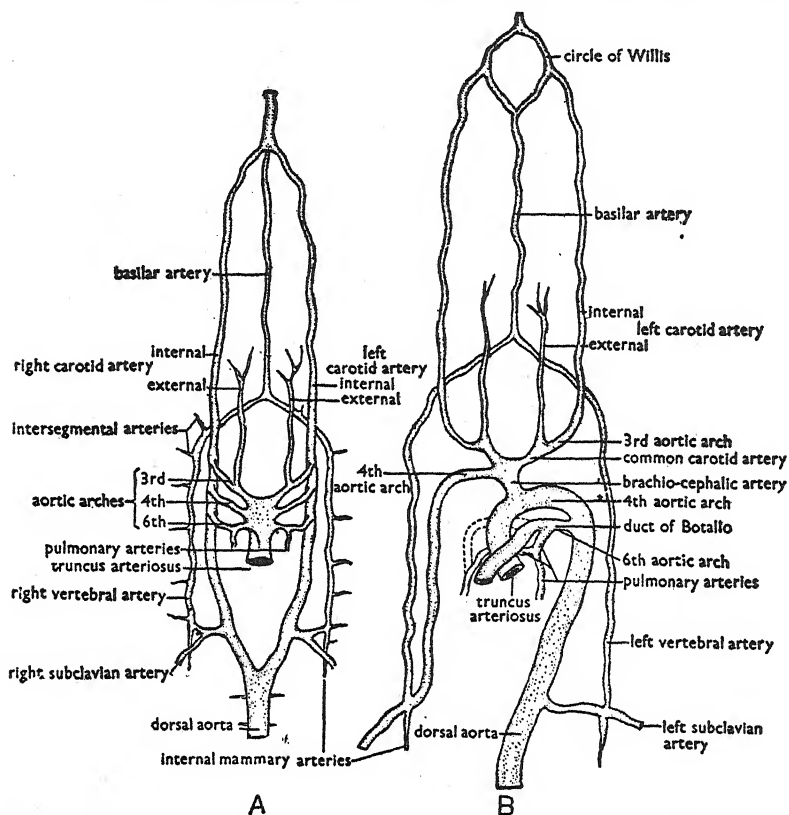


Fig. 319. — Semi-diagrammatic representation of the development of the aortic arches and other anterior arteries of the Pig. *A*. Arteries at the 10 mm. stage. *B*. Arteries of a specimen near term.

reaches the developing lung buds. These outgrowths, together with the proximal parts of the arches, constitute at the 10 mm. stage the *pulmonary arteries* (Fig. 316, *E*). It may be noted that in other Mammals studied the proximal parts of both the sixth arches continue to form a part of these arteries. In the Pig, however, as we shall see, only the proximal part of the left sixth arch persists as a part of the pulmonary system (Figs. 317, *A*, *B*; 319). Anteriorly, the first two pairs of arches

have disappeared, and each member of the third pair has given rise near its base to a new vessel. These vessels are the *external carotids*, and appear at 10 mm. as very tenuous strands extending cephalad toward the ventral part of the head (Fig. 318). Both fourth arches at this time remain well developed.

The Dorsal Aortae. — At their dorsal ends the arches of each side are connected anteriorly and posteriorly by the two *dorsal aortae*. Cephalad these aortae remain separate, and extend into the head as the *internal carotids*. Posteriorly they also continue separately at first (Fig. 312, B), but at about 6.5 mm. (17 somites) they become united at approximately the middle of the embryo to form the single *dorsal aorta*. By the 10 mm. stage this fusion has progressed to the tail, and as far forward as the anterior appendages (Figs. 316, 318).

Other Arteries Anterior to the Heart. — In the Pig and other Mammals the internal carotids are not the only dorsal arteries extending into the head. There early arise from the aorta throughout most of its length small branches between each pair of somites, the *inter segmental* (or *segmental*) *arteries*. These were also noted in the Chick. In the Pig, however, these arteries soon form antero-posterior anastomoses in the region extending from the seventh cervical somite into the head, and at the same time lose their connections with the dorsal aorta. As a result of this process there are established in the neck region anterior to the seventh cervical intersegmental arteries, a pair of longitudinal vessels called the *vertebral arteries* (Fig. 317). These arteries, however, do not continue separately clear into the head. Beneath the myelencephalon they fuse into a single median vessel termed the *basilar artery*. As regards the seventh cervical intersegmentals, it may be noted that they are starting to enlarge slightly to take part in the formation of the subclavian arteries, whose development will be described further in the next stage. The fate of the intersegmentals posterior to the seventh cervical will also be noted at that time. Meantime by the 10 mm. stage the internal carotids have each sent a branch medially to unite with the basilar, thus producing a part of the future *circle of Willis* about the hypophysis (Figs. 317, 318, 319).

Arteries Posterior to the Heart. — To complete the history of the arteries at this stage we find that somewhat caudad from the middle of the embryo, the two omphalomesenteric or vitelline arteries are among the first to arise from the dorsal aortae. These arteries connect the aortae with the vessels formed in the wall of the yolk-sac, and since the vitellines arise before the dorsal aortae have fused, they are at first

double (Fig. 312, *B*). Their function of course is to take blood from the embryo to the yolk-sac, where it receives nutriment absorbed by this organ from the uterine walls prior to the development of the allantoic placenta. At 10 mm. the aortae in the region of the origin of the vitelline arteries have fused and with them the arteries, so that a single

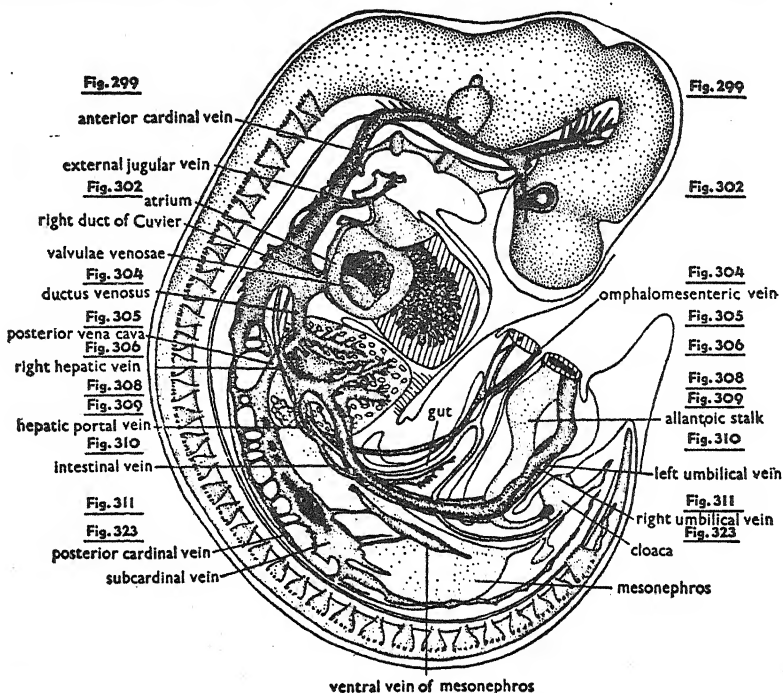


Fig. 320. — Reconstruction of a 10 mm. Pig embryo designed to show primarily the main features of the venous system at this stage. Drawing made by the same methods as used for Figs. 296 and 318. As in these figures the lines at the sides indicate where the sections denoted by the figure numbers above the lines, pass through the embryo.

vitelline artery extends along the mesentery into the body-stalk (Fig. 318). With the disappearance of the yolk-sac this vessel persists within the body as the *anterior mesenteric artery*. A short distance anterior to it the *coeliac artery* has developed at 10 mm., and extends toward the stomach region, but the posterior mesenteric artery has not yet appeared. In addition to the segmental arteries already mentioned the aorta also gives off numerous small branches at the level of the mesonephros to the glomeruli and tubules of that organ, the *renal arteries*. Lastly, so far as branches from the aorta are concerned, are the *umbilical arteries* to

the allantois. These arise quite early before the two aortae have fused in this region, and even after their fusion at 10 mm. the umbilicals remain separate. By this stage also each has produced a small branch in

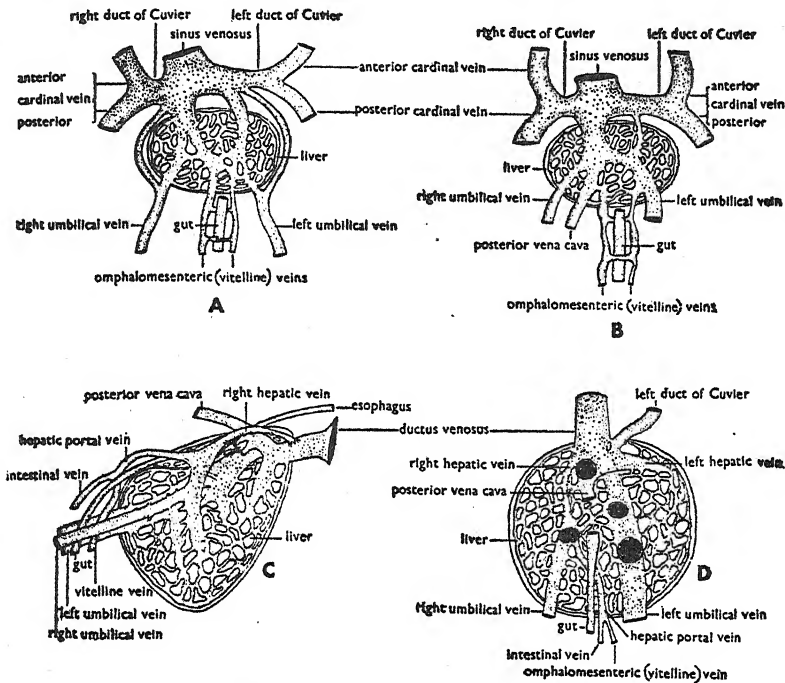
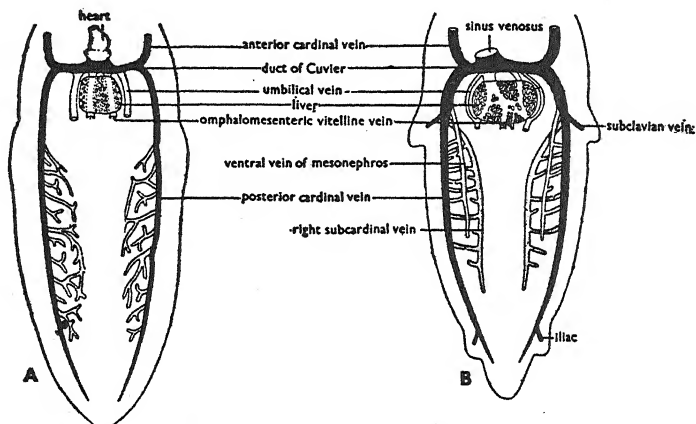


Fig. 321. — Reconstructions of stages in the development of the veins of the liver and immediate vicinity. *A.* The veins in a 5-6 mm. embryo, semi-diagrammatic. Veins in the liver according to Butler, with the omphalomesenteric (vitelline) veins extended posteriorly to show their relation to the gut. *B.* Veins in a 6 mm. Pig embryo, semi-diagrammatic. Again the vessels within the liver are according to Butler, with the omphalomesenterics posterior to it added. *C.* Veins in the liver of a 10 mm. Pig embryo viewed from the right side (enlarged from Fig. 320). *D.* Veins in the liver of a Pig at the same stage as *C*, but viewed ventrally.

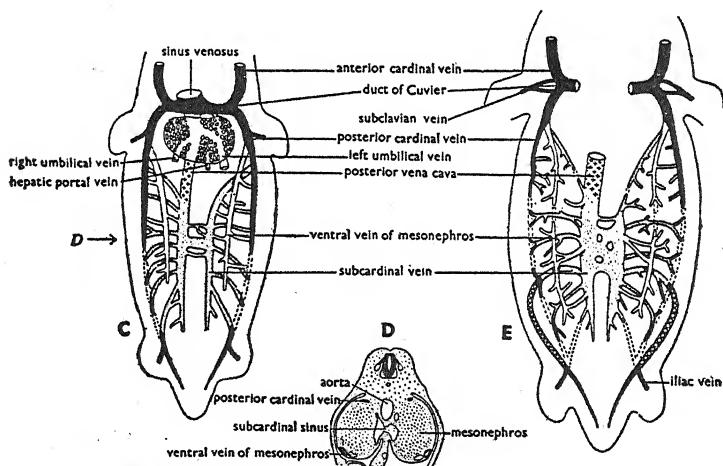
connection with the developing hind limb bud, the *external iliac*. The aorta itself continues on as a single vessel into the tail (Fig. 318).

The Omphalomesenteric Veins. — As in the Bird, among the earliest, if not the earliest, veins to develop in the Pig are the *omphalomesenteric* or *vitelline veins*. They arise just as they did in the Chick coincidentally with the formation of the cardiac tubes which fuse anteriorly to form the heart. Posterior to the region of fusion these tubes extend caudad and laterally out onto the yolk-sac where they become continuous with the capillaries and blood islands which we have noted



Early stage in any young mammalian embryo.

5-6 mm. Pig embryo.

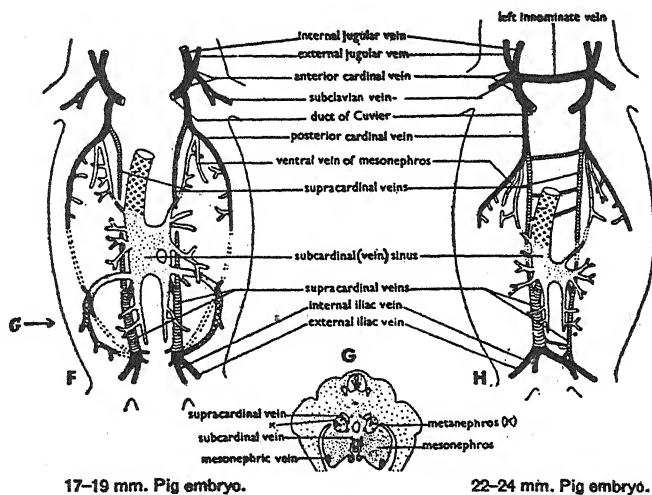


6-7 mm. Pig embryo.

12-14 mm. Pig embryo.

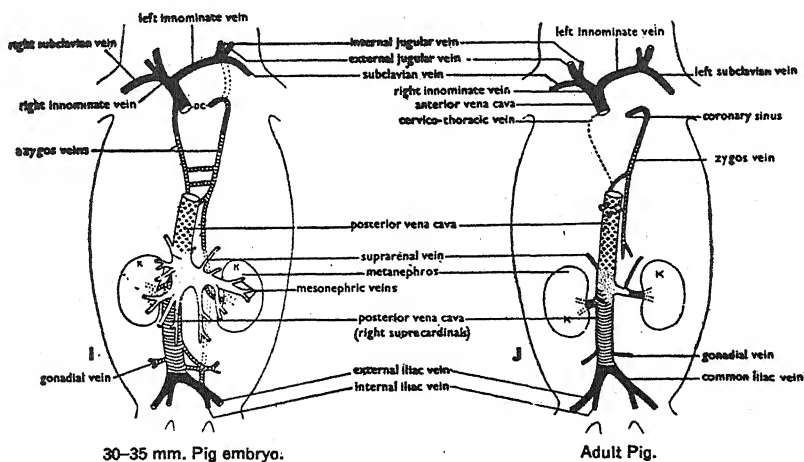
Fig. 322. — Diagrams of developing venous system posterior to heart in: A. Any very young mammal. B, C, E, F, H, I, J, in Pig at stages indicated. D. Transverse section of C at level shown by arrow.

as originating there. As development proceeds the fusion of the vitellines continues for a very short distance posterior to the atrial region of the heart to form a thin walled sac, the *sinus venosus* (Figs. 312, A; 322, A, B). At about this time also (3.5-4 mm.) the previously noted interatrial septum primum begins to develop, and in such a way that the sinus comes to open into the right atrium (Fig. 304).



17-19 mm. Pig embryo.

22-24 mm. Pig embryo.

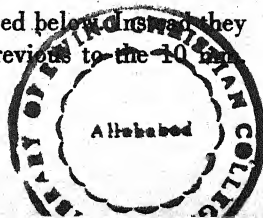


30-35 mm. Pig embryo.

Adult Pig.

Fig. 322 cont.—F, H, I, J, as noted above. G. Transverse section of F at level shown by arrow. All stages after Butler. Princeton Embryological Collection.

At this point a difference may be noted between the further development of the vitelline veins in the Chick and that in the Pig. The two veins in the Pig do not continue their fusion to form any large part of the ductus venosus as in the Bird, the major portion of that trunk arising from a different source in a way to be described below and as they remain mostly separate, within the liver and previous to the 10 mm.



stage their middle portions have broken up into a capillary network. Their anterior stumps, however, remain as the two *hepatic veins*, while their posterior parts persist for a time in the caudal half of the liver as two distinct vessels (Fig. 322, *A, B*). From there these vessels issue to pass along either side of the gut to the regressing yolk-sac. As the latter disappears they become simply two veins bringing blood from the intestine, and by the 10 mm. stage a further change has occurred, resulting in the reduction of these two vessels to the one *hepatic portal vein*. The method by which this takes place, producing the peculiar spiral course of this single vessel about the gut, is illustrated in figure 321. It involves essentially the same process as in the Chick, i.e., a fusion of the vitelline vessels first above the intestine, and then below it, with the subsequent disappearance of the left and right sides of the loops thus formed. The chief difference between the Chick and the Pig in this connection is that in the latter both sides of the loop are formed before either disappears, but as indicated the end result is the same.

The Allantoic (Umbilical) Veins. — Another pair of veins which develop very early in the Mammal are the *allantoic* or *umbilical veins*. In the Bird these are somewhat slower in forming, and it will also be recalled that at first the allantois is drained by a transitory vessel, the subintestinal vein, which opens anteriorly into the vitellines. This preliminary arrangement does not occur in the Pig. Instead the umbilical veins develop at once in essentially the same way that they ultimately do in the Bird. They arise as vessels in the lateral body wall which open anteriorly directly into the sinus venosus (Fig. 322, *A*). Posteriorly they extend around the sides of the wall, and thence via the body-stalk onto the neck of the allantois (Fig. 273). This is the situation at first, but by 10 mm. certain changes have developed as follows:

Anteriorly the two veins no longer empty directly into the sinus venosus. Instead as the liver comes into contact with the body wall, the umbilicals in that wall develop new channels connected with the hepatic capillaries (6 mm.) (Fig. 322, *B*). By the 10 mm. stage some of these capillaries in line with the flow of blood from the two umbilicals have developed into well marked channels which soon become definite vessels within the liver. The left one even at this stage is larger than the right, which soon disappears in this region. Hence the part of the left umbilical within the liver now forms the major part of the *ductus venosus*, the short anterior section which opens into the sinus, being derived from the very limited fusion of the vitellines indicated above (Figs. 320, 321, 322, *C*). Thus, as noted, the ductus has for the most part a quite differ-

ent origin from the similarly named vessel in the Chick where it arose entirely from the posterior fusion of the vitelline veins. Caudad to the liver the two allantoic or umbilical veins continue at this time to exist as separate vessels as far as the umbilical stalk, but within this stalk they have become fused into one. Thus there is but one umbilical vein in the stalk, but two umbilical arteries. Even at this stage, however, the right umbilical vein within the body wall is becoming smaller.

The Anterior and Posterior Cardinal Veins.— So far we have considered venous systems which are both intra- and extra-embryonic. It now remains to indicate the development of those veins which are entirely within the embryo. Among these the most prominent up to the 10 mm. stage are the various cardinals, whose development very closely parallels that in the Bird. Thus the *anterior cardinals* arise anteriorly on either side of the neck and head region slightly dorso-lateral to the aortae, and soon develop a capillary network connecting with the latter vessels. The *posterior cardinals* likewise develop in the same relative position to the aorta posterior to the heart. Dorso-lateral to that organ the anterior and posterior vessels of each side dip downward slightly, and join one another to form the wide, short ducts of Cuvier which slope ventrally and medially to enter the sinus venosus. A short distance cephalad to the point where the anterior cardinals enter the ducts each cardinal is joined by a ventral branch coming from the region of the mandibular arch. It is of course the future *external jugular*. Very slightly posterior to, or at its junction with, the respective duct of Cuvier each posterior cardinal receives the *subclavian* from the adjacent forelimb bud. This vein, as was the case with the corresponding arteries, results simply from the enlargement of one of the numerous *intersegmental veins* which drain into the posterior cardinals (Fig. 321, 304).

The Subcardinals and Posterior Vena Cava.— Again as in the Chick, with the development of the mesonephros the original cardinal circulation is supplemented by certain new vessels which in a 10 mm. embryo are well established. Indeed by this time the posterior cardinals have actually begun to degenerate, and their functions to be taken over by these new vessels as follows:

Along the ventro-medial border of each mesonephros a plexus of capillaries is formed (5–6 mm.), and soon these have anastomosed so as to constitute continuous vessels running the length of each mesonephros. These are the *subcardinals*, and through further mesonephric capillaries they are soon more or less connected with the posterior cardinals (Fig. 322, B). In fact anteriorly these connections presently become quite

definite and direct. Now as the mesonephroi grow the subcardinals are crowded still nearer the mid-line, and at about the middle antero-posteriorly, of the mesonephroi they fuse into a single large sinus (Figs. 311, 322, C, D, E). Into this drain all the surrounding capillaries. This comes about because, as this sinus is formed, the posterior cardinals at this level disappear entirely, though they persist for a time both anteriorly and posteriorly. Thus it happens at 10 mm. that among the capillaries draining their blood into the median *subcardinal sinus* through the mesonephros are many from the posterior parts of the posterior cardinals (Figs. 320, 322, C, E). At the same time anterior to the subcardinal sinus, the left subcardinal begins to become smaller, and to lose its connection with the anterior part of the left posterior cardinal, though this is still functioning at 10 mm. (Fig. 322, C, E). The right subcardinal, however, just as in the Bird, becomes more prominent, and at 10 mm. has affected a connection with still another new vessel. This vessel has formed from capillaries within the liver mesentery, and also from some of those within the liver itself. It is the mesenteric and hepatic part of the *posterior vena cava*, the subcardinal sinus and the anterior portion of the right subcardinal, being the other parts developed at this time (Figs. 320, 322, C, E). Anteriorly the part of the new vessel developing in the liver opens into the ductus venosus near its anterior end, where it also receives the two hepatic veins. As the caval vein grows, the anterior part of the ductus between this vein and the sinus becomes the anterior end of the vein (Fig. 321). The complete development of its posterior end will be explained in our discussion of the next stage.

In connection with the description of this vessel up to the present point, however, there is already one feature concerned with its posterior part which is becoming evident, and which merits attention. This feature is the development of a renal portal system in essentially the same way that it was formed in the Bird (Fig. 322, E). When fully developed, these systems function more or less like that of the Frog, though they arise somewhat differently, there being no subcardinals in the Frog.¹ It is interesting of course that this system exists in all these forms, yet in the Bird and Mammal is only temporary. It is perhaps even more remarkable that it is always the right side (in the Bird and

¹ It appears that in the Pig, and very probably the Bird, not so much of the blood coming from the posterior of the embryo is actually supplied to the mesenteric tubules as in the Frog. Instead more of it seems to be routed more directly through the organ, while the tubules, as well as the glomeruli, are supplied more from arterial sources.

Mammal the right subcardinal (in the Frog the right posterior cardinal) which enters into the formation of the posterior vena cava. Such facts can scarcely be entirely coincidental.

One minor feature regarding the cardinals in the 10 mm. Pig which differs from that in the Chick should be mentioned to avoid confusion. In the Chick there are no other vessels than those just described. In the Pig, on the other hand, some of the capillaries along the ventro-lateral side of each mesonephros also anastomose to form a small vessel extending antero-posteriorly along this region. It is called the *ventral vein of the mesonephros*, and since it also connects through capillaries with the respective posterior cardinal, it might be mistaken for a subcardinal. Its smaller size and superficial ventral position, however, distinguishes it and it soon disappears (Figs. 320, 322, C, D, E).

The Pulmonary Veins.—One other important intra-embryonic venous system which has no relation to the cardinals, but which also starts to develop at an early period is the *pulmonary*. Since the pulmonary arteries have been seen to arise as early as the 7.5 mm. stage, the development of the veins at about that time might be anticipated, and they have in fact arisen. There is some question, however, as to just how these vessels have been formed, e.g., whether as an outgrowth from the atrium, or as in so many other cases, by an anastomosing of plexuses along their course. In any event they exist at this stage as small veins which proceed from each lung bud, and unite in a common trunk which enters the left atrium. Later as in the case of the arteries the pulmonary veins also suffer certain alterations which will be noted in due course.

THE URINOGENITAL SYSTEM

Although these systems are ordinarily considered together because of the close association of some of their parts both embryologically and anatomically, it is convenient as previously, to describe their development separately. We shall begin with the excretory system since it is the first to become clearly evident.

THE EXCRETORY SYSTEM

The Pronephros.—In the Pig, as in the Bird, there is a gesture made toward the development of a *pronephros*. On each side its rudimentary tubules arise as usual from the intermediate mesoderm, and occur in the cephalic region from about the sixth to the fourteenth somites. These vestigial organs are of course without functional significance, but

the tubules turn and grow caudad to give rise to the *pronephric ducts*, in the way with which we are already familiar. By 10 mm. all parts of this system, save the ducts, have virtually disappeared.

The Mesonephros. — The *mesonephros* arises in the intermediate mesoderm from about the fourteenth to the thirty-second somite of the Pig. As usual it first appears as spherical concentrations in this mesoderm, three or four such concentrations being developed opposite each somite. These form vesicles, and the vesicles produce tubular outgrowths which become coiled, and open into the old pronephric, now *mesonephric, duct*. The vesicular portion of each tubule is invaginated by the usual knot of capillaries forming a *glomerulus*, supplied with blood by branches from the aorta, and draining into tributaries to the subcardinal veins. The invaginated part of the tubule of course constitutes *Bowman's capsule*.

Anteriorly the mesonephric duct is often difficult to distinguish in cross section from the numerous mesonephric tubules, but more caudally it can generally be located along the ventral border of the organ. Posterior to the mesonephros this duct continues to the cloaca, and by the 6 mm. stage has entered it. By 10 mm. the antero-ventral region, into the sides of which this entrance was affected, is beginning to be separated from the postero-dorsal part by the urorectal fold in the manner already described (Fig. 337). Thus the ducts are coming to open into the part of the cloaca termed the urinogenital sinus which is in the process of being added to the neck of the bladder (allantois). These arrangements in the cloacal region are the beginnings of changes which will ultimately bring about fundamental differences between conditions in these parts in the Bird and the Mammal. These differences will be discussed in detail later in connection with the development of the external genitalia. At this time, however, the most striking peculiarity of the mammalian excretory system lies in the remarkable relative size of the mesonephroi themselves. Thus in a 10 mm. Pig these organs are far larger than at any period in the Chick, being in fact much the largest structures in the embryo (Figs. 296, 310, 311). The functional significance of this difference is not known.

The Metanephros and Ureter. — As the student is already aware, the mesonephric kidney in all Amniotes is ultimately replaced by a third or metanephric kidney. This kidney starts to appear at the 5-6 mm. stage as a very small diverticulum growing out from the postero-dorsal side of each mesonephric duct just dorsal to the point where these ducts enter the cloaca. By 10 mm. the diverticula still issue from

the mesonephric ducts rather than the neck of the bladder, but have grown anteriorly somewhat, and the cephalic portion of each is enlarged slightly. The enlarged portion represents the lining of the future *pelvis* of the kidney, and is already surrounded by a concentration of intermediate nephrogenic mesoderm (Figs. 296, 323). This meso-

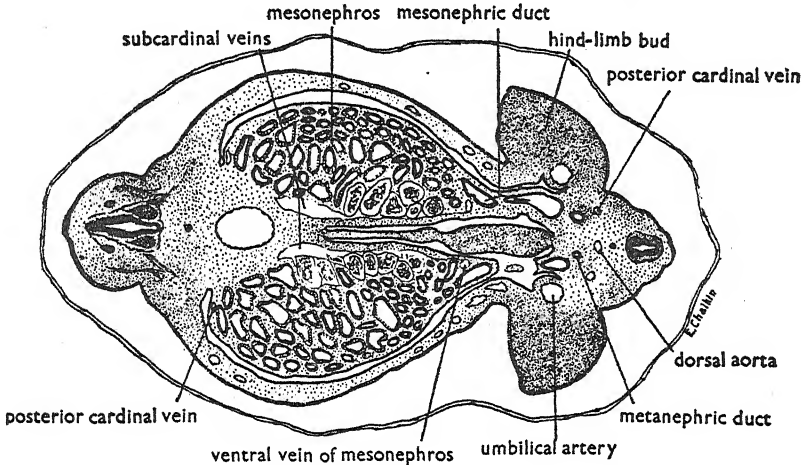


Fig. 323. — Transverse section through the region of the mesonephros, umbilical arteries, mesonephric and metanephric ducts and hind limb buds of a 10 mm. Pig. See reconstruction Figs. 296, 318, 320.

derm is carried forward with the pelvic portion, and later furnishes the material from which the kidney tubules are made. The remainder of the outgrowth of course becomes the future metanephric duct or *ureter*.

THE GENITAL SYSTEM

The Gonads. — These are barely in evidence at the 10 mm. stage. They may sometimes be detected, however, as very slight thickenings on the medial sides of the mesonephroi, somewhat anterior to the middle.

T

HE LATER DEVELOPMENT OF THE PIG

HAVING completed our descriptions of the Pig embryo as a whole, and of the various systems at the 10 mm. stage (20–21 days), we are now prepared to indicate the further development of this animal as far as it is profitable to carry it. This means in most instances, either to the adult condition, or to a condition near enough to it so that the steps required to attain the adult state are quite obvious. As in the discussion of the earlier development we shall begin by a consideration of external features.

The Flexures. — Following the 10 mm. stage the Pig embryo gradually straightens to some extent. This process first involves mainly the dorsal flexure (15–20 mm., Fig. 324), and later the cervical and lumbosacral flexures. As in other vertebrates the cranial flexure is permanent, but since it concerns chiefly the brain it also becomes less obvious externally as development proceeds.

External Features Posterior to the Head and Neck Region. — At 15 mm. the boundaries of the somites are still clearly visible, and the milk ridge has become evident. By 20 mm. the somite markings have pretty much disappeared, while along the lower border of the milk ridge five or six mammary anlagen are present. Ventral to these anlagen in both these stages the abdomen protrudes greatly, due to the developing mass of viscera within it. By the 50 mm. stage, however, these have been drawn up, and the ventral contour is about that of a well-fed adult. Throughout all these periods there has been relatively little growth of the umbilical cord. Its diameter does ultimately increase, however, due to growth of the contained blood vessels and connective tissue, so that at term it measures from 8–10 mm., while the length of the whole animal may be as much as 25–30 cm. The paddle-like appearance of the feet at 10 mm. has been referred to, and this condition still prevails at 20 mm. By that time, however, the existence of five toes in each foot is clearly in evidence, and the limb joints are slightly suggested. In the Pig and other Artiodactyls, as is well known, the first digit (homologue of the thumb or great toe in Man) soon vanishes entirely. The third and fourth digits develop evenly to form the cloven hoof, while the second

and fifth digits remain short and more or less vestigial. This condition is well advanced in an embryo of 40–50 mm.

The Head and Neck Regions.—Probably the most striking changes of all in any mammalian embryo are those connected with the head and neck, especially with relation to the face, and we shall now indicate these changes in their main outlines.

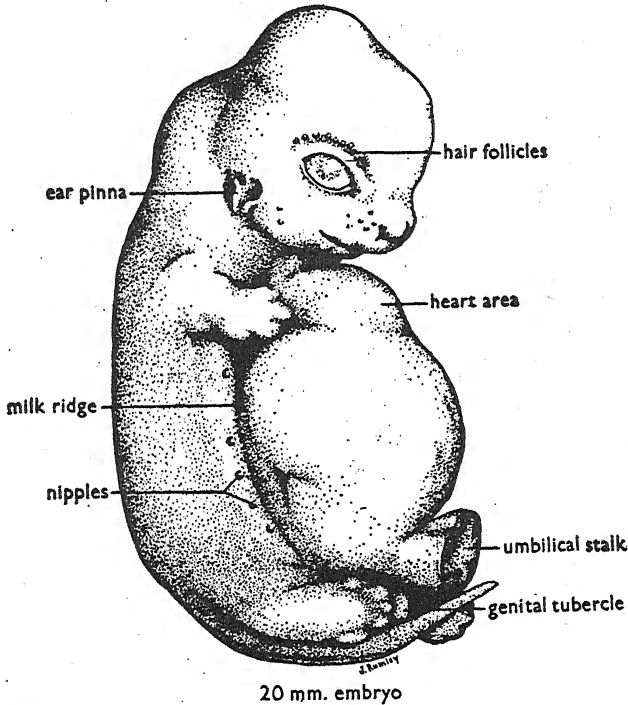


Fig. 324. — A 20 mm. Pig embryo viewed from the right side.

When last described at 10 mm. it will be recalled that there were four visceral clefts and four arches visible in a side view, the first arch being the mandibular (Fig. 294). Also apparent were the maxillary processes and nasal pits. Each pit was bounded laterally by a naso-lateral process which was separated from the adjacent maxillary process by a groove running from oral cavity to eye, the lachrymal groove. Viewed anteriorly (Fig. 295) the frontal process separated the nasal pits, and adjacent to each pit this process was thickened to form the naso-medial processes. Reference to the appropriate figures makes evident the great similarity of these facial anlagen in a 4–5 day Chick and

a 10 mm. Pig. It may now be added that the resemblance between Pig and Man at comparable stages is even closer. Indeed the latter are so much alike not only with regard to facial features, but in other respects, that to a casual observer the differences between a 10 mm. Pig embryo and a 10 mm. Human embryo would be scarcely noticeable. The changes which gradually ensue to produce the condition in the head of the adult Pig will now be indicated.

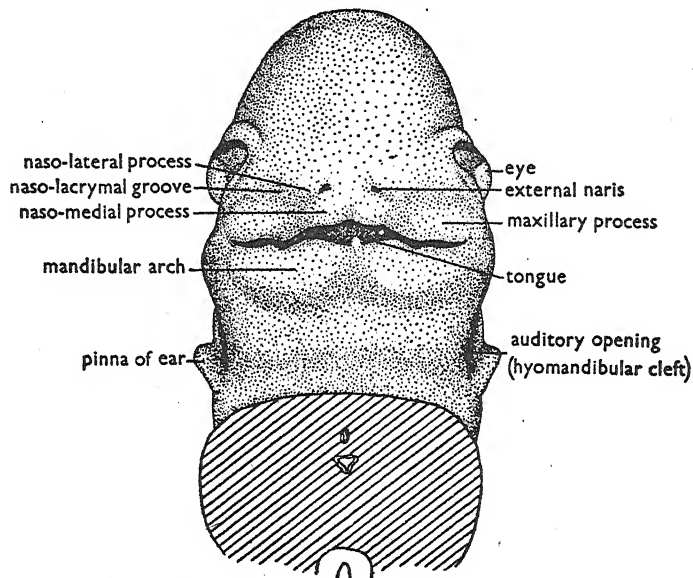


Fig. 325. — A view of the face of a 17 mm. Pig embryo from the antero-ventral side.

The *lower jaw*, it may at once be noted, is derived entirely from the mandibular arches which grow antero-medially until they meet. Posteriorly they form an angle with the maxillary processes which constitute the larger part of the *upper jaw*. However, these latter processes do not meet one another anteriorly, and hence do not form the antero-medial part of this jaw. Instead this part is comprised of the naso-medial processes whose forward extremities grow together. In so doing they crowd the original median region, i.e., the frontal process, backward (Figs. 295, 325). Thus the naso-medial processes come to form the *pre-maxillary* part of the upper jaw, and the *nasal septum*, while the frontal process forms only the *nasal bridge*. While this fusion between the naso-

medial processes is occurring in the mid-line, each of these processes is also fusing postero-laterally with the respective maxillary process, and also with the respective naso-lateral process. These fusions serve to bound the nasal pits antero-laterally, and cut them off from the edge of the oral cavity, thus producing the *external nares* (Fig. 325). Posteriorly these pits break through into the oral cavity, and so give rise to the temporary *internal nares*, of which more will be said in connection with the development of the mouth proper. While the bridge of the nose is formed as noted from the frontal process, its sides (*alae*) are constituted by the naso-lateral processes. Also the lachrymal groove separating these processes from the maxillary processes is closed over so as to form a tube, the *lachrymal duct* connecting eye and nose.

Further development of the Pig's face consists largely of the outgrowth of all these parts. Indeed the whole procedure from 10 mm. onward may be roughly pictured thus: It is much as though all the above processes were approached from the front by invisible fingers which grasp these processes, squeeze them together, and then draw them out anteriorly to make the Pig's snout. Essentially these same changes occur in the development of the human face from the same original parts, except that, fortunately from our point of view, the "drawing out" procedure is not carried to such an extreme. It is of some interest to note in this connection that a failure in the fusion of the naso-median processes with the respective maxillary processes on one side or both results in the formation of the defect known as "harelip." An inspection of Figure 325 will show why this is true.

On the sides of the head the almond shaped eyes do not possess lids, even at 20 mm., though the follicles of the coarse bristles constituting the Pig's eyebrow are clearly visible. Both upper and lower lids appear very shortly, however, at about 24 mm., as folds of skin. Eventually these folds meet and fuse so that the eye is completely covered for a time, and in some animals this condition even persists for a while after birth, e.g., in the Cat, in which case the animal is said to be born "blind." As regards the eye itself, it has previously been indicated that one prominent difference between the Chick and the Mammal is the fact that in the earlier stages the eyes of all mammalian embryos are definitely smaller than those of comparable Bird embryos. This is still true at the stage of the latter corresponding to that of the 20 mm. Pig, and it may be further remarked that the Pig eye is even smaller relatively than that of many other Mammals, e.g., Man or Rat.

THE NERVOUS SYSTEM

In the preceding chapter the development of the nervous system was carried to the point characteristic of a 10 mm. Pig, and in so doing it was found convenient to treat it by parts. These involved the brain, the

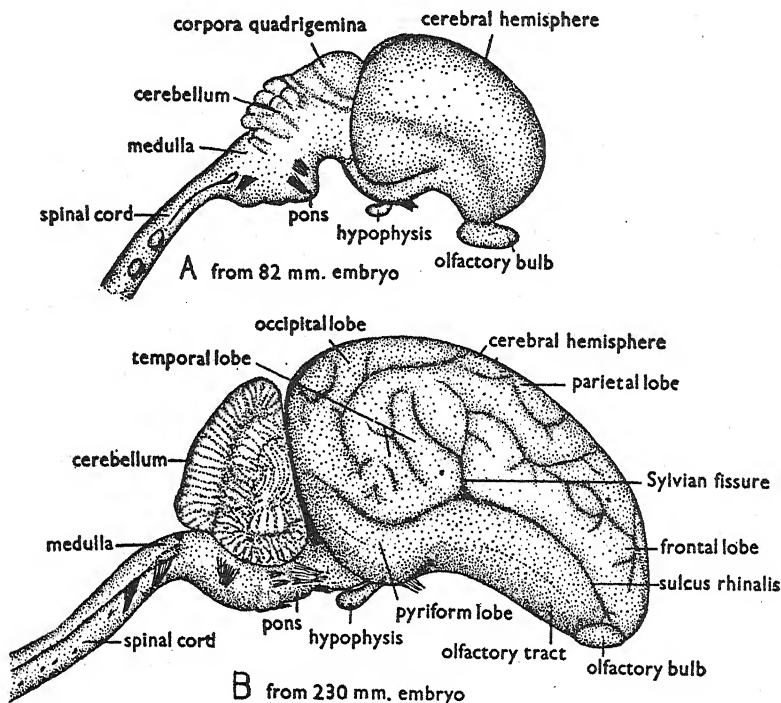


Fig. 326. — Lateral views of two stages in the development of the Pig brain. In *B* the corpora quadrigemina are entirely covered by the cerebrum and cerebellum.

neural tube, the cranial nerves, the spinal nerves and the organs of special sense. We shall now proceed with the further development of these parts so far as seems profitable.

THE BRAIN

The Telencephalon. — This structure is of course the anterior part of the prosencephalon which is separated from the posterior part (diencephalon) by the same boundaries already familiar in the Chick. As previously noted it has already started to give rise to its most important and conspicuous products, the *cerebral hemispheres*. As in the Chick

these antero-lateral outgrowths contain cavities, the *lateral ventricles*, which communicate with the small remaining space within the telencephalon by the *foramina of Monro*. This latter space as usual constitutes a small part of the anterior portion of the *third ventricle*.

It was noted in the discussion of this region in the Chick, that although the cerebral hemispheres are relatively prominent structures in that form, they never attain the size and complexity characteristic of the Mammal. In the latter animal their size eventually causes them to constitute by far the larger part of the brain, and to cover entirely the mammalian homologues of the Bird's conspicuous optic lobes. In addition to their mere size, in the Pig and most other higher Mammals, their surface area (cortex) is increased by complex foldings, the narrow depressions or fissures between the folds being known as *sulci*. It should now be noted that one of the more conspicuous of these sulci extends horizontally along the ventro-lateral region of each hemisphere, serving to separate the upper portion, or *neopallium*, from the lower portion or *rhinencephalon*. It is therefore called the *sulcus rhinalis*. Other sulci within the neopallium serve to divide it into the *frontal*, *parietal*, *temporal* and *occipital lobes* or regions, which in turn are still further subdivided (Fig. 326). The rhinencephalon does not contain conspicuous sulci, but does give rise at its anterior extremity to the *olfactory lobes* or *bulbs*, while its lateral walls constitute chiefly the *pyriform lobes*. Quite evidently the rhinencephalon is phylogenetically the older part of the telencephalon, while the neopallium is a recent addition reaching its most conspicuous development in Man.

The Diencephalon. — This posterior portion of the prosencephalon, whose laterally compressed cavity comprises most of the *third ventricle*, has already been noted as giving rise to the optic vesicles and infundibulum. The connection of the optic stalks with this part of the brain is marked as usual by the optic recess which really constitutes the ventral boundary between telencephalon and diencephalon. Immediately posterior to this recess and hence definitely in the wall of the diencephalon, is a thickening which, as in the Bird, is the *optic chiasma*, within which eventually the fibers of the optic nerves cross each other. Adjacent to the chiasma on the posterior side (i.e., the floor) occurs a thin region of wall termed the *lamina post optica*, and immediately beyond that the pouch-like *infundibulum* presently makes contact with *Rathke's pocket* growing antero-dorsally from the stomodaeum. As previously indicated these two latter structures together produce the adult *pituitary* or *hypophysis*. The anterior part of this organ, compris-

ing the *pars distalis*, *pars intermedia* and *pars tuberalis*, is derived entirely from Rathke's pocket, while the posterior part forming the *pars nervosa* and the stalk are derived entirely from the infundibulum.

Upon the anterior side, i.e., the roof of the diencephalon, two structures appear. The more posterior, or really dorsal, is an outpushing whose lumen later becomes occluded, and which develops into the *epiphysis*. Anterior to this the rather thin roof of the third ventricle becomes invaginated, and this invagination divides into two parts which extend forward into each lateral ventricle. These invaginations or folds are partially produced and augmented by the development of blood capillaries within their walls, and they thus come to constitute the *anterior choroid plexus* or plexuses.

The sides of the diencephalon are eventually thickened to form the *optic thalami*, the thalami of each side being connected by a median fusion of the walls. The transverse band of tissue formed by this fusion is called the *massa intermedia*.

The Mesencephalon. — As previously indicated, the roof of this region, which in the Bird forms mainly the optic lobes, in the Mammal gives rise to the *corpora quadrigemina*. As the name suggests, these consist of four, instead of two, thickened outpushings which, as already noted, are well covered in the adult by the large cerebral hemispheres. The more anterior pair are apparently more or less homologous in function with the avian optic lobes, and might be so named, but are not. Instead they are called the *superior colliculi*. The posterior pair are centers for auditory reflexes, and hence might be referred to as auditory lobes, but again their actual names are the *inferior colliculi*. The sides and floor of the mesencephalon become greatly thickened by fiber tracts connecting the anterior and posterior parts of the brain. In the Bird they were designated as the *crura cerebri*, though this term is not so commonly employed in the Mammal. Here these regions are often referred to as *peduncles*. At all events the growth of these parts compresses the lumen of this region of the brain into a narrow canal connecting the third and fourth ventricles, and universally termed the *aqueduct of Sylvius*.

The Rhombencephalon. — It will be recalled that in the Mammal, as in the Bird, the posterior part of the brain, i.e., the rhombencephalon, is early divided into two parts, the anterior metencephalon and the posterior myelencephalon. The former is the shorter region, and indeed consists primarily in its dorsal aspect of the thickened sloping roof of the posterior side of the isthmus fold (Fig. 297). As in the Chick this dor-

sal region presently undergoes extensive growth to form the *cerebellum*, a part of the brain especially concerned with muscular coordination. The division of this organ into a median lobe, the *vermis*, and lateral lobes, which appeared to some extent in the Bird, is still further emphasized in the Mammal, and in addition each lobe develops extensive foldings (Fig. 326). Ventro-laterally beneath the cerebellum the walls of the metencephalon are greatly thickened by fiber tracts, partly from fibers originating in the cerebellum itself, and partly from fibers passing through these walls to and from anterior parts of the brain. In this region, as in the mid-brain, the thickenings so caused are often designated as peduncles. The ventral thickening becomes so pronounced eventually as almost to comprise a sort of reversed flexure. It is called the *pons*, and because of the effect just indicated is sometimes referred to as the *pontine flexure* (see the Chick). Beside the thickenings caused by the fiber tracts there is also at deeper levels the development of numerous neurones connected with the cranial nerves which arise from the sides of this part of the brain. The lumen of the metencephalon remains fairly large, and is considered a part of the *fourth ventricle*.

Posterior to the metencephalon the myelencephalon becomes a tube which tapers off into the spinal cord, and is designated as the *medulla*. In most respects the medulla resembles the cord except that it is wider, especially anteriorly, and its extensive roof consists of a thin membrane into which blood capillaries soon press. This produces a vascular infolding similar to that described in connection with the diencephalon, and in this case termed the *posterior choroid plexus*. The broad shallow cavity of this region into which these folds push is also quite extensive, and constitutes the larger part of the fourth ventricle. The ventro-lateral walls of the medulla are essentially similar to what has already been described with respect to the walls of the neural tube. They consist internally of a lining of ependymal cells, a middle mantle layer of neuroblasts which become nerve cells, and an outer marginal layer of fibers. It may be further noted that dissection, or cross sections, show that a groove runs along either side of the internal wall of this region, termed the *sulcus limitans*, dividing it into a dorsal and ventral part.

THE NEURAL TUBE

When last noted at 10 mm. the essential layers and types of cells in the tube were already beginning to differentiate. Further development consists mainly in the continued production and differentiation of these cells, so that the cord not only becomes larger, but assumes its charac-

teristic shape. Thus in cross section we find the ependymal cells lining the now relatively small central canal, and sending their supporting processes transversely through the substance of the cord. Within the mantle layer the spongioblasts ultimately all become supporting cells of other types, while the neuroblasts all finally become transformed into nerve cells. As a result of growth this layer finally assumes in cross section a somewhat butterfly shape (i.e., with wings extended), constituting the so-called gray matter of the cord. The dorsal and ventral extensions (horns) of the "butterfly wings" serve to divide the outer marginal layer into four tracts of relatively white material. These tracts or columns consist of bundles of medulated fibers, the myelin substance in the fiber sheaves giving the tracts their white appearance. The dorsal column consists mainly of sensory fibers conducting impulses to the brain, while the two lateral columns and the ventral column are motor paths from the brain to the various spinal nerves.

THE CRANIAL NERVES

The origins of all cranial nerves, save the I and II, have already been indicated, and there is little more that need be said about them except to note briefly the parts which they ultimately innervate in the Pig. In general the relationships of nerves and parts are the same as in the Chick in so far as comparable structures exist. Thus the III or oculomotor nerve as usual supplies the inferior oblique, and the superior, inferior and internal (anterior) rectus muscles of the eye. The IV or trochlear nerve innervates the superior oblique eye muscle, while the external (posterior) eye muscle is innervated by the VI or abducens nerve. Passing to the most anterior of the mixed nerves we find that the ophthalmic branch of the V or trigeminal nerve comes to supply the snout, eyeball, and upper eyelid; the maxillary branch supplies the upper lip, jaw, palate, face and lower eyelid; the mandibular branch supplies the tongue, lips, muscles of the jaw, the lower jaw itself, and the external ear. The VII or facial nerve was but slightly developed at 10 mm. As its name suggests, it supplies the face, and is primarily motor, though the existence upon it of the geniculate ganglion shows that it contains some sensory fibers. These fibers come eventually to join the mandibular branch of the V nerve and evidence indicates that they concern the sense of taste. The VIII is of course the auditory nerve, and is entirely sensory, being concerned with both hearing and the sense of equilibrium. Though at first closely associated with the VII its ganglion later becomes more distinct, and eventually divides into two parts the

vestibular ganglion and the *spiral ganglion*. The branch from the former supplies the semicircular canals, is termed the *vestibular nerve*, and is concerned with equilibrium. The *cochlear nerve* from the spiral ganglion innervates the cochlea, and is concerned with hearing. The IX or glossopharyngeal nerve fibers are mainly sensory, and come to supply the pharynx and tongue. Such motor fibers as there are pass to the pharynx. The X or vagus nerve develops further as follows: Sensory fibers from the ganglion jugulare come to innervate the external ear, while sensory fibers from the ganglion nodosum eventually reach the pharynx, larynx, trachea, esophagus and thoracic and abdominal viscera. Motor fibers of the X nerve innervate the pharynx and larynx, while other motor fibers connect with the sympathetic ganglia, and supply the visceral musculature. The XI or spinal accessory nerve, as previously noted, loses Froriep's ganglion (which disappears), and thus this nerve becomes entirely motor, and its fibers are very closely associated with the motor fibers of the vagus. Many of them also run to sympathetic ganglia, and thence to the viscera. Other motor fibers of this nerve help to innervate the pharynx and larynx, while still others originating along the cervical region of the cord proceed to the trapezius and sterno-cleido-mastoid muscles. The XII or hypoglossal nerve is the motor nerve of the tongue. The muscles which it innervates originate further back and migrate anteriorly as the tongue develops, carrying the branches of the XII nerve along with them. Indeed phylogenetically the tongue muscles are probably derived from the occipital myotomes, and the XII nerve was originally a spinal nerve which has recently become cranial.

The origin and development of the I and II cranial nerves will be taken up in connection with the organs of special sense along with which they develop.

THE SPINAL NERVES

The Somatic Nerves. — As regards the further development of the somatic spinal nerves, it may be said that their afferent and efferent fibers grow until they come in contact respectively with skin or muscle. Then as the latter parts develop and move further away the fibers grow so as to maintain their contact. The sheaths of these fibers have two sources. The *neurilemma* is formed of cells of ectodermal origin which accompany the fibers as they grow out. The *myelin sheath* on the other hand is not itself cellular; but is a cell product which accumulates at numerous points between the neurilemma and the nerve fibers. These

accumulations then spread until they meet, the meeting points forming the *nodes of Ranvier*.

The Autonomic Nerves. — The origins of the autonomic nervous system have already been stated, and the fact that it involves both parasympathetic and sympathetic parts. Each part of course has to do with controlling the involuntary movements of the viscera, and as in the case of the somatic nerves, when the fibers make contact with the organs which they are to innervate they grow with them. It is of interest that the two parts of the system largely overlap with respect to the structures which they reach, and that they have opposing functions. Thus the sympathetic fibers reaching the heart from certain postganglionic neurones carry accelerating nerve impulses. On the other hand, impulses in the parasympathetic fibers from the brain via the vagus nerve to postganglionic neurones on the organ itself, have a retarding influence.

THE ORGANS OF SPECIAL SENSE

THE OLFACTORY ORGAN AND I NERVE

Following the formation of the olfactory pits, and the establishment posteriorly of their communications with the oral cavity, the further development of the olfactory organs proceeds as follows: In the lateral walls of each nasal chamber folds develop known as *conchae* or *naso-turbinals*, these folds being more numerous in many lower animals and in the human fetus than in the human adult. Meanwhile the epithelium, at first simple cuboidal, soon becomes more or less stratified columnar throughout a large part of its extent, with the occurrence of many ciliated and goblet cells. On the more dorsal conchae, and on the median septum formed by the fusion of the naso-median processes, however, the original cuboidal epithelium becomes transformed into that of the specifically olfactory type. In these regions no goblet cells are formed, and the tall columnar cells which develop here lack cilia. Also just beneath the surface certain of the cells turn out to be neuroblastic. From each of these a fine bristle-like process projects through the epithelium to the surface. At the same time from its opposite pole each of these cells sends an axone to the olfactory bulb or lobe of the brain. The bundle of axones from each of the two olfactory areas then come to constitute the I or *olfactory nerves*. Eventually the various *nasal sinuses*, i.e., the ethmoid, maxillary and frontal are developed by the invasion of the bone by the non-olfactory nasal mucosa which gradually excavates the bone substance, and then lines the spaces so formed. The further development

of the posterior nasal passages and the internal nares will be referred to in connection with the account of the oral cavity.

THE EYE AND OPTIC NERVE

Except for one feature the development of this important organ is essentially the same in the Mammal as in the Bird, where it was described in some detail. The vascular pecten, presumably an organ aiding in the nutrition of the inner parts of the Bird eye, does not exist in the eye of the Mammal. There are, however, blood vessels of course which supply the mammalian retina and lens. These are capillaries arising from a branch of the ophthalmic artery. This branch enters the optic cup along the groove on the ventral side of the optic stalk by way of the proximal part of the choroid fissure. It is at first called the *hyaloid artery* because it supplies only the developing lens, but later it supplies the retina also, and is then called the *central artery of the retina*. Shortly after it appears, axones from the cells of the neuroblasts (future ganglionic) layer of the retina start growing back along the artery which they soon come to surround. As the number of these fibers increases they encroach on the tissue of the original stalk. Finally they become medullated and surrounded by a connective tissue sheath, while the old stalk cells are virtually eliminated. Thus are produced the I or *optic nerves*. As is well known, in the case of the mammalian eye the fibers from the median sides of the two retinas cross in the optic chiasma, while those from the lateral sides do not.

As suggested the development of the eye proper, aside from the points noted, is so similar to that of the Chick that no further comment on it is deemed necessary.

THE AUDITORY ORGAN

The Membranous Labyrinth. — In the 10 mm. Pig the only indication of the auditory organ was the occurrence of the usual otic vesicle with its upgrowing endolymphatic duct. It now remains to state that from this vesicle the *membranous labyrinth* of the inner ear develops essentially as in the Bird, except that in the Mammal one feature of it develops considerably further. Thus it will be recalled that in the former case the semicircular canals arise from the upper part of the otocyst termed the utricle. Then the lower portion of the otocyst partly constricts away, and produces an outpocketing called the sacculus. Up to this point the situations in the Bird and Mammal are similar. In the Bird, however, it will be remembered that the larger part of the ventral

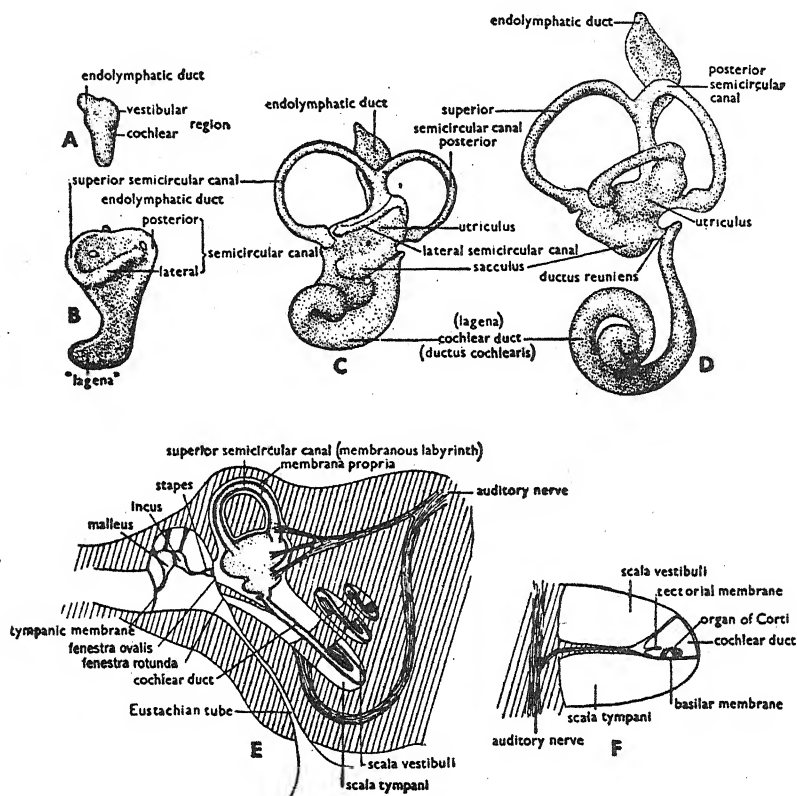


Fig. 327.—A, B, C and D, stages in the development of the membranous labyrinth of the Human ear. After Streeter. Although this is the Human ear and not that of the Pig, the latter is presumably very similar, as are those of all Mammals so far as known. All views are of the left ear from the left, i.e., lateral, side. A. The otic vesicle from a 6.6 mm. embryo, showing rudiments of the membranous semicircular canals starting to form, also the beginning of the endolymphatic duct. B. Membranous labyrinth from a 13 mm. embryo. C. Membranous labyrinth from a 20 mm. embryo. D. Membranous labyrinth from a 30 mm. embryo. E. A semi-diagrammatic representation of the middle and inner ear opened from the side. Modified from various sources. F. A diagrammatic section through one side of the cochlea, including of course the scala tympani and vestibuli and the cochlear duct, showing the organ of Corti.

portion of the otocyst is not involved in the sacculus, but grows out into a relatively short tube termed the lagena. In the Mammal these same parts exist, but here the whole "lagena" is called the *ductus cochlearis* or *cochlear duct*, and its connection to the utricle becomes narrowed to a slender tube, the *ductus reuniens*. Furthermore the remainder of the mammalian ductus cochlearis continues to grow until it has produced

an extensive spiral tube on whose floor the cells eventually become rearranged and differentiated to form the *organ of Corti*, and the *tectorial membrane*. These last named structures, the most elaborate parts of the organ of hearing, have no counterpart in the Bird. This, it may be suggested, is a somewhat remarkable fact in view of the auditory stimuli which some members of this latter group can produce, and hence presumably appreciate. Surely the song of the Nightingale should require a more complicated organ of reception than the Pig's grunt! Finally it remains to state that, as in the Chick, the whole membranous structure derived from ectoderm is closely covered by a mesenchymal layer, the *membrana propria* (Fig. 327).

The Bony Labyrinth. — Again as in the Bird, there has been developed around the membranous labyrinth and its mesenchymal *membrana propria* a *bony labyrinth*, the two labyrinths being separated by the *perilymphatic space*. Naturally, however, in this case the bony capsule or labyrinth has also to be more elaborately formed in order to encase the spiral ductus cochlearis. Not only does it also become spiral in order thus to encase this region, but in doing so it becomes divided into two channels. One, dorsal to the ductus cochlearis, is the *scala vestibuli*, while the other ventral to it is the *scala tympani*. At the apex of the spiral, at the end of the ductus cochlearis these channels communicate. At the other end surrounding the sacculus and the utricle the wall of the *scala vestibuli* contains the *fenestra ovalis* to whose membranous covering is attached a bone of the middle ear. The wall of the *scala tympani* in this region contains the *fenestra rotunda* also covered by a membrane.

The Middle Ear. — Considering next the middle ear we find again the same parts involved as in the Chick, but once more with a slightly different outcome in certain respects. The first or hyomandibular pouch grows out until it makes contact with the ventral part of the corresponding visceral furrow. This initial contact, however, does not long continue. The upper part of the pouch enlarges, but at the same time withdraws somewhat from the ectoderm of the furrow, while between them mesenchyme develops. Presently within this mesenchyme cartilaginous concentrations arise, representing the developing ear bones or ossicles. In this case, however, instead of there finally developing only one such bone, the columella, three of them appear — the *malleus*, *incus* and *stapes* (Fig. 327). At the same time that the cartilaginous anlagen are becoming ossified to form these bones, the mesenchyme surrounding them is being absorbed. As this occurs, the upper end of the visceral

pouch once more extends so that it surrounds the developing ossicles, including a little of the disappearing mesenchyme. It also again almost reaches the outer ectoderm, being separated from it only by a thin sheet of mesenchyme. Thus there is formed the permanent cavity of the middle ear, or *tympanic cavity*. The part of the visceral pouch between this cavity and the pharynx remains, of course, as the *Eustachian tube*. It thus also comes about that the *tympanic membrane* or *tympanum* consists, as in previous cases, of tissue derived from each of the germ layers, the outer lining being ectodermal, the middle layer mesodermal, and the inner lining endodermal. On its median side the lining of the tympanic cavity is in contact with the bony capsule of the inner ear, and so forms a membrane over each of its two fenestra. To the membrane covering one of these, the fenestra ovalis, the stapes is attached, while at the other end of the bony chain the malleus of course is fastened to the tympanum. Though most of the mesenchyme about the ear bones is ultimately absorbed, some of it becomes differentiated into the small muscles attaching the ossicles to the wall of the tympanic cavity. It is also interesting to note that in Man this mesenchyme does not entirely disappear until a few months after birth. This apparently serves to prevent free movement of the ossicles, and thus to protect the ear of the infant from too strong stimulus by loud noises.

Homologies.—Turning now to the possible homologies of the mammalian ear bones, it will be well to recall the situations which were described in the Frog and Chick. Thus in the former animal, though only one bone, the columella, finally existed as a separate entity within the completed middle ear, there were originally two elements concerned. For, fused to the inner end of the columella, there was also the operculum, lying within the fenestra ovalis. At its outer end, moreover, the columella connected with a ring of cartilage around the tympanic membrane called the annulus tympanicus. In the Chick there was again a columella which fused with an opercular element, in that case called the stapes, but the annulus tympanicus was lacking. In these cases it was suggested that the columella was possibly the homologue of the hyomandibular element of the hyoid arch of the primitive fishes, and that the annulus tympanicus might be the homologue of the palato-quadrata cartilage of such forms. In the Mammal, where there are three separate ossicles, the question of possible homologies again arises. It has been suggested that the mammalian stapes corresponds to the columella, and hence ultimately to the hyomandibular, the incus to the palato-quadrata (primitive upper jaw) and the malleus to Meckel's car-

tilage (primitive lower jaw). This obviously leaves the opercular element of the Frog and the stapes of the Chick quite out of the picture. As stated in connection with the Frog, there is good evidence, experimental and otherwise, to support these suggested homologies, and they are, therefore, quite generally accepted. Thus the intriguing notion that parts once connected with the coarse work of seizing food have finally been promoted to the delicate "white collar" task of transmitting sound waves, seems to be well established. It probably affords an example of functional adaptation correlated with a changing environment.

THE DIGESTIVE AND RESPIRATORY SYSTEMS

The Oral Cavity.—Originally the anlage of the oral cavity existed merely as the stomodaeum, a relatively shallow pocket lined with ectoderm. By the 10 mm. stage, the oral plate which constituted the stomodaeal union with the fore-gut had broken through, and the roof of the stomodaeal cavity had given rise to Rathke's pocket. Subsequent to 10 mm. the stomodaeum becomes greatly deepened to form the actual oral cavity, while Rathke's pocket becomes separated from it, and as already noted, gives rise to the anterior part of the pituitary. The deepening of the cavity as just suggested is extensive; so much so in fact, that eventually we find the tonsils occurring at about the original site of the oral plate. This enlargement is brought about chiefly by the outgrowth of the mandible, and the various processes giving rise to the face, nose and upper jaws. The external aspects of this procedure have already been described, but it remains to indicate some of the details more especially concerned with the mouth itself. Thus it will be recalled that the maxillary processes formed the sides of the upper jaw (*maxillae*), while the anterior tip was derived from the fused naso-medial processes. This tip is the *premaxillary* region, and from it there grows backward a small median plate constituting the more anterior portion of the palate, and termed the *median palatine process* (Fig. 328). By far the larger part of the permanent roof of the mouth, however, is formed by the two lateral plates, the *lateral palatine processes*. These are simply median extensions of the maxillary processes which soon meet and fuse in the middle line. The more posterior plate so formed then unites with the median palatine process and thus together these parts constitute the complete *hard palate*. It is now to be recalled that the temporary internal nares open into the oral cavity through its original roof fairly near the front. The formation of this new roof beneath the first one, however, creates a new chamber between the two roofs into which the nares open.

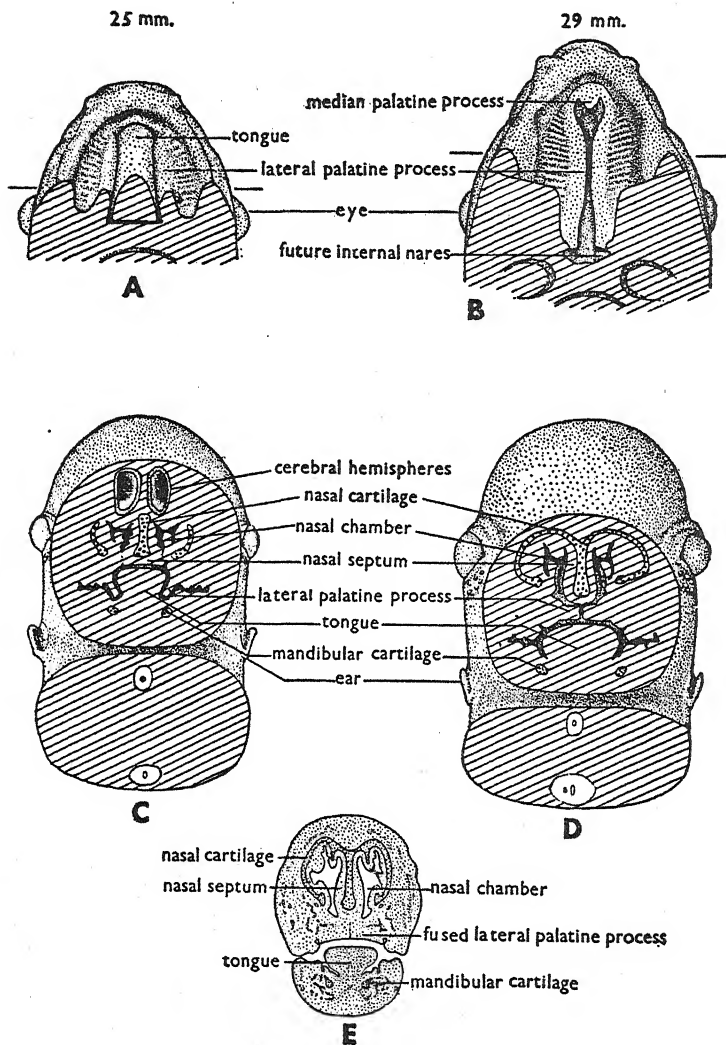


Fig. 328. — Illustrations to show the development of the roof of the mouth and the nares of the pig. *A* and *B*. The roofs of the mouths of specimens of the sizes indicated, the lower jaw having been removed. *C* and *D*. Transverse sections of the snouts of the same specimens at the levels indicated by the lines at each side of *A* and of *B*. *E*. A transverse section, made with a microtome, of a snout of a somewhat older embryo than *D* at about the same level. This section appears somewhat smaller than *D* because it does not show the surrounding parts of the head, and because it was apparently somewhat compressed laterally in cutting.

The further development of the nasal septum to fuse with the new or lower roof then divides this chamber into two lateral parts. In this way there is produced essentially a posterior extension of the nasal cavities

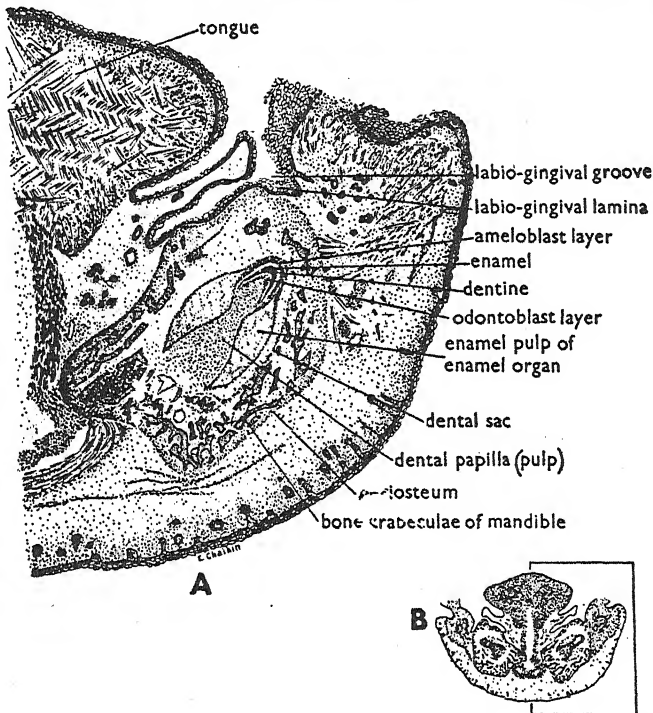


Fig. 329. — *A*. Transverse section through the right side of the lower jaw and tongue of a Pig embryo somewhat older than the oldest in Fig. 328, showing the beginning of tooth development. *B*. The same section shown in *A*, but at a much lower magnification so as to show the whole jaw, with an indication of the part from which *A* was taken. Connection of enamel organ with dental ledge has gone.

so that the definitive internal nares eventually open well back toward the throat.

While this is going on in the roof of the mouth, the *tongue* is being formed in the floor. As in the Chick it is made up of three thickenings, a median one called the *tuberculum impar*, and a pair of lateral ones. These lateral primordia soon overgrow the median one to form a single mass which for a time lies between the lateral palatine processes. As these come together, however, the tongue drops down to its adult position (Fig. 328).

Finally by the 28-30 mm. stage a thickening of the oral epithelium (ectoderm) has developed around the border of both jaws. This thickening, termed the *labio-dental ledge* or *lamina*, pushes into the underlying mesenchyme, and presently its inner and outer edges become particularly developed. The outer edge or part is called the *labio-gingival lamina* (later a groove), and serves to separate the lip from the inner part of the originally single thickening (Fig. 329). This inner part is called the *dental ledge* or *lamina*, and within it the teeth eventually develop. Since these latter structures do not occur at all in modern Birds, and were not mentioned in the Frog where they are not highly evolved, we shall consider their formation separately along with that peculiarly mammalian product, hair.

The Pharynx.—The pharynx begins at approximately the line where the oral plate disappears, and thus is the most anterior part of the alimentary and respiratory tracts to be lined by endoderm. It is also the part which is flanked laterally by the remains of the visceral arches posterior to the mandibular, and by the pouches. These arches and pouches very shortly disappear as such, but as will be apparent, their remains give rise to various adult structures as follows:

Thus the second or hyoid pair definitely produce the *styloid processes* and *lesser horns* of the *hyoid*. There is also the possibility, as noted, that the columella (mammalian stapes) of the ear may be derived from it. The third pair of arches give rise to the *greater horns* of the *hyoid*, while from the fourth pair of arches is derived the *thyroid cartilage* of the larynx. No distinct fifth arches are ever visible, in the Pig, but from the region where they should lie come the *cricoid* and *arytenoid cartilages*. All of these parts are of course involved in the formation of the *larynx*, and immediately adjacent structures.

Turning to the products of the visceral pouches we find that, as we have already noted, the first or hyomandibular pouches take part in the formation of the Eustachian tubes and tympanic cavities. The second pair in connection with ingrowths of lymphoid tissue produce the main or *palatine tonsils*. The third pair give rise to the main or definitive thymus bodies (thymus III), which migrate posteriorly until they are eventually located in the upper part of the thorax. It is interesting to note that in the Guinea Pig the thymus bodies are permanently in the neck instead of the thorax. This is apparently because the third pouches in this case are so firmly fused to the ectoderm that they cannot be carried backward (Klapper, '46). In addition to becoming transformed into thymus tissue the third pouches also produce outgrowths which be-

come the chief pair of parathyroids (parathyroid III). These are located in the neck where they are ultimately associated closely with the posterior parts of the thyroid. With respect to the fourth pair of visceral pouch derivatives there has been some disagreement. So far as the Pig is concerned Godwin ('40) concludes that, as noted, this pair of pouches are not always present. When they are, he thinks that the remains of the pouches proper become incipient thymus bodies (thymus IV) which later disappear. In addition there are produced in this animal two distinct outgrowths either from the pouches if they are present, or if they

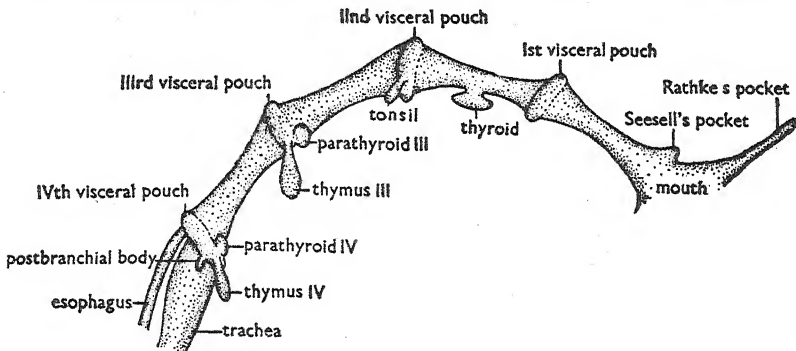


Fig. 330. — The pharyngeal region of a 10 mm. Pig embryo, showing diagrammatically the regions from which the thyroid, thymus and parathyroid bodies either have been, or will be, derived.

are not, from the region of the pharynx where they would be. One of these outgrowths is an additional pair of parathyroids (parathyroid IV), each of which, according to Godwin, soon divides into two parts which persist. Others, however, have claimed that they disappear. The other outgrowths are the pair of post-branchial bodies. Each of these bodies eventually becomes embedded in the thyroid gland. According to Godwin, however, there is nothing to indicate that they ever become actual thyroid tissue as believed by some (Fig. 330).

The *thyroid gland* as in other forms arises as an evagination from the floor of the pharynx between the first and second visceral pouches. It soon loses its connection with the pharyngeal floor and becomes almost, though not quite, completely divided into two lobes (Fig. 296). These lobes then migrate posteriorly somewhat to lie eventually at the base of the neck. As noted the parathyroids are closely associated with the thyroid, and the ultimo-branchial body becomes imbedded in it, whether a part of it or not. Though the thyroid becomes separated from its point of origin this point at the future root of the tongue is marked, in Man

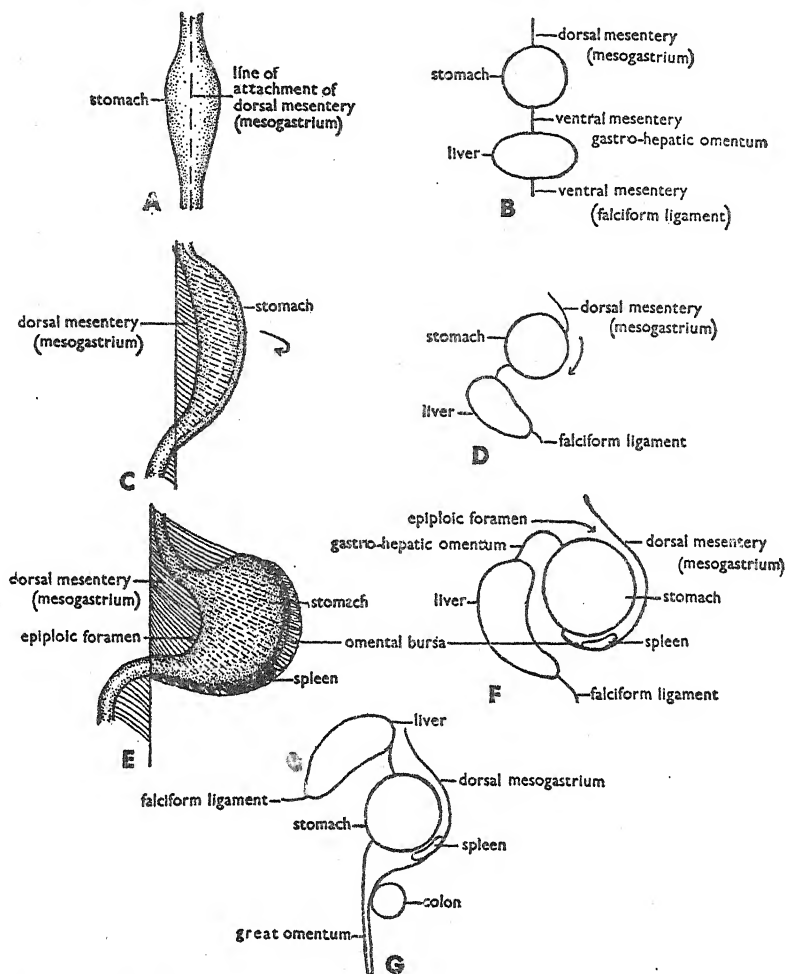


Fig. 331. — *A*, *C* and *E* are semi-diagrammatic representations of the developing stomach and mesenteries of the Pig, as viewed from the ventral side. The dash lines in *C* and *E* represent the part of the mesogastrium on the dorsal side which is covered by the stomach in this view. The liver and ventral mesenteries (gastro-hepatic omentum and falciform ligament) are not shown in these figures as they would obscure the stomach. *B*, *D* and *F* are diagrams of transverse sections through *A*, *C* and *E* viewed from the anterior. *G* is a diagram of a transverse section of liver, stomach and colon in Man at a later stage when the stomach and colon have become transverse to the body. Hence this section is mid-sagittal for the body as a whole. The great omentum, which does not occur in the Pig, is obviously an extension of the fold of the original dorsal mesentery down across the anterior (ventral) wall of the abdomen. It is largely this fold which accumulates fat in older persons.

at least, by a permanent depression, the *foramen caecum*. The histological differentiation of the thyroid is fairly simple. The endodermal derivatives become broken up into nests of cells which form the secreting follicles, surrounded by mesodermal connective tissue and blood capillaries.

One other structure of the pharynx remains to be mentioned, the *epiglottis*. It arises as a thickening in the floor of the pharynx just posterior

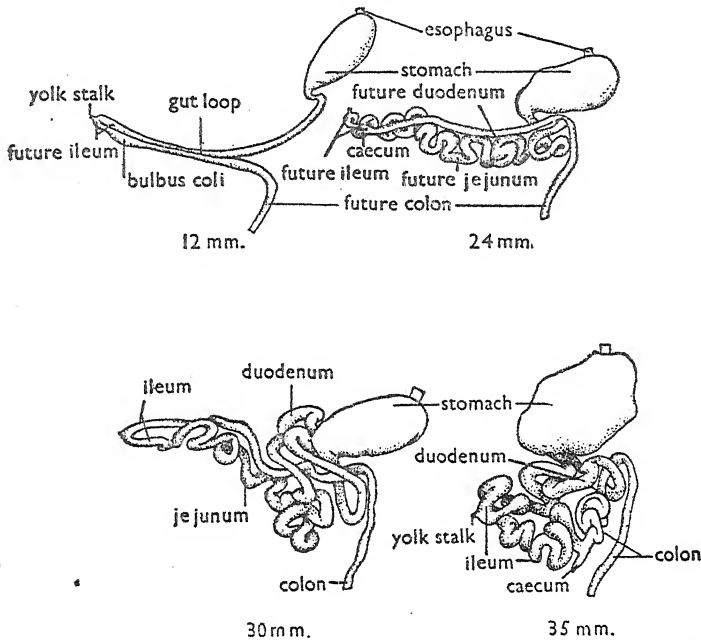


Fig. 332. — Stages in the development of the intestine of the Pig from the gut loop stage to that in a 35 mm. embryo. After Lineback.

to the lower ends of the third pair of visceral arches. It grows posteriorly, and eventually overhangs the slit-like opening to the larynx, i.e., the *glottis*.

The Esophagus. — At the back of the pharynx the original gut canal had become separated at 10 mm. into a dorsal and ventral division, and the latter was starting to become differentiated into the respiratory system. The dorsal part, on the other hand, was already becoming narrowed to constitute the esophagus. In carrying on the description of these parts it will be convenient to discuss the digestive portion of the originally undivided gut separately from its respiratory derivatives. In so doing we shall consider the former first.

The esophageal part of the digestive tract posterior to the pharynx is, as previously indicated, already relatively constricted. Its inner endodermal lining becomes differentiated into a smooth non-ciliated epithelial layer, and into mucous glands which extend into the connective tissue (submucosa) beneath the epithelium. The connective tissue and muscular coats are of course derived from mesoderm.

The Stomach and Its Mesenteries. — At 10 mm. the stomach was represented by an enlargement in the primitive gut posterior to the esophagus. As elsewhere this part of the gut was attached to the dorsal body wall by its dorsal mesentery (*dorsal mesogastrium*). This en-

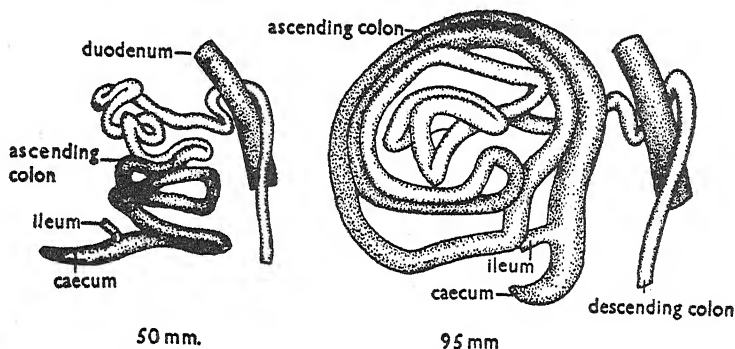


Fig. 333. — A continuation of the development of the Pig intestine shown in Fig. 332 with special reference to the region of the colon. After Lineback.

larged region is already slightly bent with the convex side dorsal, and very shortly three things happen to it. (1) The bend increases, (2) the anterior end shifts to the left, and (3) the whole structure rotates on its longitudinal axis in a clockwise direction when viewed from the esophageal end. As these movements take place it is obvious that some adjustment must be made by the attached mesenteries. What occurs is that the dorsal mesogastrium is extended to accommodate the bending and rotation of the stomach. Furthermore, since the line of attachment of mesentery to stomach does not change as the stomach rotates, this line necessarily rotates with it. Thus in the new position the line of mesenteric attachment simply follows the curve around the left convex side of the organ. As these changes occur with respect to the dorsal mesentery, the ventral mesentery has likewise had to shift its position so that it now leaves the stomach on the concave side of the latter (Fig. 331).

In connection with these alterations certain further facts need now to be noted as follows. We have seen how, as the stomach changes its

position, the dorsal and ventral mesenteries change to accommodate it. In the course of this accommodation it is clear that the dorsal mesentery must increase in extent. It remains to add, however, that this mesentery increases more than would be required by the shift of the stomach. As a result a fold of the mesentery comes to extend out beyond the stomach so as to form a sort of wide pocket. This fold and pocket are called the *omental bursa*, the *spleen* later developing within the walls of the fold. Inspection of Figure 331 will show that an opening from the general coelom into this more restricted pocket area occurs from one side. This opening, at first quite wide, becomes much narrowed later on, and is known as the *epiploic foramen*. In Man the fold itself also develops further to form still another structure which will be noted in connection with the development of the intestine.

The Intestine. — The intestine at 10 mm. consisted anteriorly of a short region to which the liver and pancreas were attached, the duodenum, followed by a loop whose limbs passed into and out of the umbilical stalk. At the ventral apex of this loop a very narrow tube still represented the yolk-stalk, while the upper end of the posterior limb bent around caudally to the rectum (Fig. 332, 12 mm.). The whole structure was of course supported by a mesentery. By the 24 mm. stage the anterior limb of the former simple loop has become very markedly coiled, and it is this region which forms the main part of the *small intestine*. Upon the posterior limb of the loop a short distance from the apex, a slight outpocketing or caecum was evident at 10 mm., and shortly thereafter it becomes a distinct diverticulum (Fig. 332, 24 mm.). In Man this caecum gives rise to a finger-like extension, the *vermiform appendix*. From the point where the caecum grows out the distal part of the original posterior loop becomes the large intestine or *colon*. Eventually this part bends so that the small intestine enters it at a right angle. Also it too becomes coiled, forming a loop, a condition not found in Man (Fig. 333). In correlation with all this bending and coiling the dorsal mesentery of these parts of the intestine also becomes thrown into somewhat involved configurations which it is not necessary to go into. It is of interest, however, to note a further development of the mesentery in the region of the stomach which occurs in the case of Man, but not in the Pig. It occurs as follows:

The fold of the bursa, as previously described for the Pig continues subsequently to increase in extent in the human embryo, and to grow caudad, until eventually it comes into contact with the parts of the colon occupying a transverse position in Man. When this condition is reached

the bursal fold fuses with the peritoneal covering of the colon, and later, after birth, continues to grow still further in a caudal direction. At the same time the two limbs of the fold beyond the line of fusion with the colon unite with one another to form a double sheet. This sheet, the *great omentum*, thus constitutes a sort of apron covering the lower abdominal viscera on their ventral (anterior) side between them and the ventral body wall (Fig. 331). This is possible because in this region the ventral mesentery has long since disappeared. Later this part of the omentum usually becomes a storage place for fat, a feature which is frequently all too obvious in older men and women.

The Rectum.—At the 100 mm. stage the cloaca, into which the large intestine opens, was in process of being divided by the urorectal fold to form the rectum and the urogenital sinus. The cloacal membrane also had not yet ruptured. The completion of these processes, however, is more readily described in connection with the description of the development of the external genitalia and related parts. It will therefore be deferred until that subject is discussed.

The Liver and Its Mesenteries.—We are now prepared to return to the development of the outgrowth of the duodenum. It will be recalled that in the Pig there is only one hepatic diverticulum instead of two. This single outgrowth (ductus choledochus), moreover, had produced several anteriorly directed buds, the anlagen of the liver tubules, while the remainder of the outgrowth was extending posteriorly as the anlage of the cystic duct and gall bladder (Fig. 307). This anlage rapidly elongates to form the definitive duct while its end enlarges to produce a bladder. Meanwhile the anteriorly directed tubules grow out into the ventral mesentery where they soon come into contact with the vitelline (omphalomesenteric) veins into which they push. They thus break these vessels up into innumerable sinusoidal capillaries which ramify amongst the liver tubules. In this manner the tubules and capillaries come to constitute the main mass of the hepatic substance with only a relatively small amount of supporting connective tissue. Having completed our description of the development of the organ itself it remains to say a few words regarding its mesenteries.

It has been repeatedly stated that the liver develops within the ventral mesentery of the stomach and duodenum. It may now be added that the part of this mesentery which attaches the hepatic mass to the intestine and stomach is known as the *lesser omentum*, or sometimes the *gastro-hepatic omentum* (gastro-hepatic ligament in the Chick). Beneath the liver, i.e., between it and the ventral body wall, a small portion of mes-

entry also permanently persists in the Mammal, where it is termed the *falciform ligament*, connecting liver and body wall. This ligament is absent in the Bird as previously noted (Figs. 331, 335).

The Pancreas. — Even as the liver in the Pig has only one origin instead of two, so the Pig pancreas has only two origins instead of three. The two primordia in question were already in evidence at 10 mm. One consisted of an outgrowth from the dorsal side of the intestine of a mass

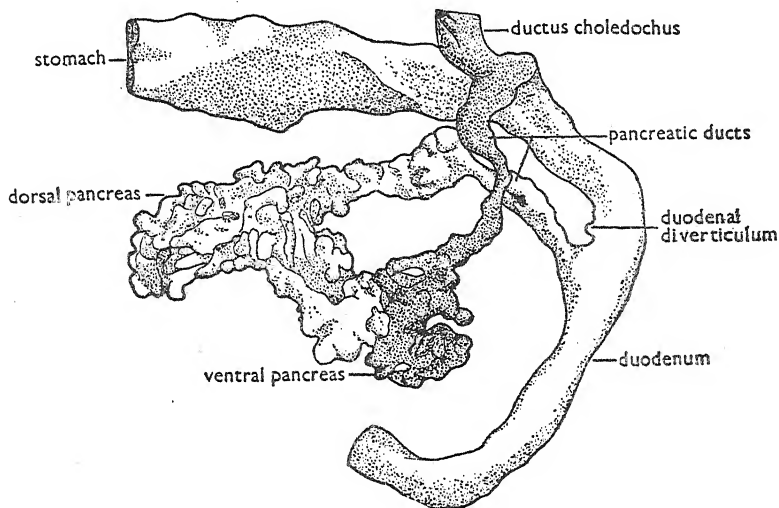


Fig. 334. — Later development of the dorsal and ventral pancreas. Slightly modified from Thyng.

of cords at a level slightly caudad to the origin of the ductus choledochus. The other arose from the ventro-lateral side of the duct itself (Fig. 307). The two growing masses soon fuse, and the cords of which they consist become tubular. These in turn produce numerous buds which develop into one of two things. Part of the buds remain connected with the tubules, and form the *pancreatic acini* which produce digestive secretions. The remaining buds become segregated, and constitute among the tubules little aggregations of highly vascularized tissue, the *islets of Langerhans*. Although the pancreas in the Pig has two origins as indicated, the adult organ has only one duct. This is derived from the dorsal outgrowth, and hence connects directly with the duodenum. The ventral connection with the ductus choledochus in this case disappears (Fig. 334).

It is of interest to note at this point that in the Mammals generally

632 THE LATER DEVELOPMENT OF THE PIG

this double, rather than triple, origin of the pancreas is the common procedure. Whether one or both primordia are to persist as ducts, however, and if only one, which one, varies in different animals. Thus in the Horse and Dog there are two permanent pancreatic ducts. In the Sheep and Man on the other hand there is only one, and in these cases the ventral one opening into the base of the common bile duct. In the Ox, and in the Pig (as already indicated), however, the dorsal duct is the persistent one, opening as noted into the duodenum.

Lastly, it should be recalled that as the liver outgrowths occur into the ventral mesentery, so the pancreatic outgrowths push into the dorsal mesentery. Furthermore, though they start into this mesentery at the level of the duodenum, the fused pancreatic elements soon extend anteriorly into that part of the mesentery supporting the stomach, i.e., the mesogastrium. Then later as this forms the omental bursa we find the pancreas in the more dorsal limb of the bursal fold, which eventually becomes adherent to the dorsal wall of the coelom (Fig. 331).

The Respiratory System.—The cartilages of the larynx have already been noted in connection with the fate of the visceral arches. Also the initial development of the trachea and bronchial outgrowths were indicated as present at 10 mm. Following this period the main bronchial tubes and their branches continue to push out into the coelomic spaces (*pleural cavities*) beneath the esophagus and above the heart (Fig. 303). The lining of the tubules is columnar or cuboidal, but at their terminals the tubules produce little sacs, the lung *alveoli*, and here the epithelium becomes thin and flat.

It must now be pointed out that when these endodermal outgrowths first occur they do not really lie in the pleural cavities. Rather they lie in a thick sheet of mesoderm which hangs from the dorsal body wall like a mesentery, and which, in addition to the trachea and lung buds, also contains the esophagus. It is the dorsal part of the *mediastinum*. Though within this structure at the start, the branching bronchi, as indicated, soon push out of it into the antero-lateral extensions of the coelom termed the pleural canals or cavities. As they do so they carry, reflected over them, a layer of mesoderm. This produces the mesothelium of the *visceral pleura*, the connective tissue about the alveoli and bronchi, and the cartilaginous rings of the bronchi.¹ At the roots of the lungs the mesothelium is of course reflected laterally onto the

¹ It has been claimed (Clements, '38) that the endodermal epithelium of the alveoli in the Pig (and probably other Mammals) later disappears entirely, leaving the blood capillaries covered only by a very thin sheet of connective tissue.

outer wall of each pleural canal to form the *parietal pleura*. Finally it remains to note that the pleural (coelomic) spaces within which the lungs lie are not at first separated posteriorly from the rest of the coelom. This and the completion of the pericardium comes about in a manner which will now be described.

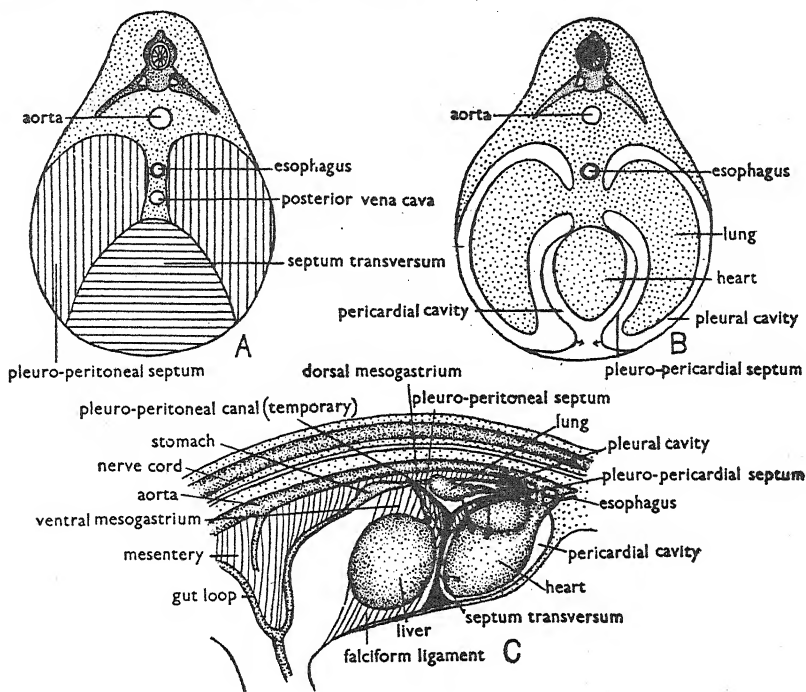


Fig. 335. — Diagrams to illustrate the separation of the pleural, pericardial and abdominal cavities, and the formation of the diaphragm in the Pig and other Mammals. *A.* Transverse section of the body just behind the septum transversum. *B.* Transverse section of the body through the lung region. *C.* Lateral view of median region showing forming septa in relation to heart, liver, lungs and gut.

COMPLETION OF THE DIVISION OF THE BODY CAVITY

The Diaphragm. — The development of the pericardium and diaphragm has already been described somewhat in the case of the Bird where, however, the strictly diaphragmal parts are incompletely formed. Also the structures involved are somewhat different in their origin. We shall therefore start from the beginning in the Pig.

The first part of the diaphragm to appear is the *septum transversum*. In this case it consists of a layer of tissue growing dorsad from the ven-

tral body wall just anterior to the liver to whose face the septum is fused. The median part of this septum also forms the posterior wall of the pericardial cavity, i.e., the part of the *parietal pericardium* separating the cavity from the coelom posterior to it. The sides of the septum, however, form the ventro-lateral parts of the diaphragm separating the ventral portions of the pleural cavities from the coelom posterior to them. The dorso-lateral parts of the diaphragm completing this separation are formed by a pair of membranes, the *pleuro-peritoneal folds*, growing out from the body walls (Fig. 335, *A*). In the middle they meet the dorsal mediastinum and complete the diaphragm. These folds also extend anteriorly in such a way as to bound the pleural cavities (canals) ventrally and the pericardial cavity dorsally. The ventral and caudal growth of the lungs then occurs, causing these organs to lie more on either side of the heart than above it. As this takes place the lungs split off more and more of the pleural-peritoneal folds from the body walls, and push these augmented folds before them. As this occurs on the median side next to the heart, the folds come to constitute the lateral and ventral as well as the dorsal pericardial wall, and likewise the medial pleural walls. Hence these parts of the pleural-peritoneal folds (septum) are called the *pleuro-pericardial septum* (Fig. 335, *B, C*).

The posterior pericardial wall formed by the median part of the septum transversum has already been noted. Anteriorly where the vessels of the heart emerge, the parts of the parietal pericardium come together, and are reflected over the heart muscle as the *visceral pericardium*. Here also these parts fuse to form the *dorsal mesocardium*, attached to what was the ventral edge of the dorsal part of the mediastinum. It is to be noted, however, that though the pleuro-pericardial folds meet and fuse ventrally, the pleural cavities never become co-extensive. Hence the ventral wall of the parietal pericardium is attached to the ventral body wall. Thus the pericardium and heart now form a central mass connecting the former ventral edge of the dorsal part of the mediastinum with the body wall. This mass might then be referred to as the ventral part of the mediastinum. Actually because of shifts during development the various parts of the mediastinum are differently named, but the details of this need not be gone into here.

THE CIRCULATORY SYSTEM

When this system was previously discussed we began with a description of the blood islands, and followed with the development of the heart, leaving the intra-embryonic blood vessels until last. Nothing fur-

ther need be said of course about the blood islands which soon disappear, and for various reasons it is more convenient to begin with the blood vessels rather than the heart. We shall therefore start with the arteries.

THE ARTERIES

The Aortic Arches and Related Vessels. — It will be recalled that at 10 mm. the first pair of aortic arches had disappeared, while the third, fourth and sixth remained, the fifth being vestigial. From the base of the third pair the external carotids were just beginning to develop, while the sixth pair had produced rudimentary pulmonary arteries. Dorsally the arches on each side were still connected by the dorsal aortae which continued anteriorly as the internal carotids. Posteriorly the aortae had fused as far forward as the anterior appendages, and posteriorly to the tail.

Subsequent to 10 mm. we find that the base of each third arch between the origin of the respective external carotid and the point of origin of the fourth arch becomes lengthened somewhat. These lengthened bases thus come to constitute the two *common carotids* (Fig. 317, *B, C*). Continuing posteriorly the part of each dorsal vessel between the third and fourth arches as usual disappears, while on the left side the fourth arch and the dorsal aorta posterior to it enlarge and persist as the main or *great aortic arch* of the adult (Fig. 319, *B*). At this point two important differences between Bird and Mammal are to be noted. One of course is the fact that in the former it was the right arch which so persisted. A second difference is that whereas in the Bird the fourth arch opposite the great aorta entirely disappeared, in the Mammal it does not. Thus in the Mammal this arch, in this case the right, remains to form two things. Its proximal part constitutes the *brachiocephalic artery* (innominate) while its more distal parts, together with a portion of the right dorsal aorta, comprise the proximal part of the *right subclavian artery*. The rest of the right dorsal aorta disappears. The *left subclavian*, it may be noted, arises directly from the distal part of what was the left dorsal aorta, but which later becomes simply a part of the main aortic arch. The genesis of the right subclavian distal to its aortic portion will be referred to presently. It now remains to add in connection with the carotids that in the Pig the left common carotid usually shifts its point of attachment so that eventually it does not arise directly from the left (main) aortic arch. Instead it emerges from the brachiocephalic close to the right common carotid (Fig. 319).

Passing now to the sixth aortic arches we are familiar with the manner in which they take part in the formation of the pulmonary arteries in the Frog and Chick. It has been indicated also that this same situation occurs at first in the Pig (Fig. 316, E). Subsequent to 10 mm., however, certain changes occur which are a little different from events in the Chick, or in other Mammals. Thus in the case of the Pig the two pulmonary branches which proceed from the upper parts of the sixth arches to the lungs, fuse with one another in their proximal regions. This single branch then retains the connection with the left sixth arch, but loses the connection with the right sixth which disappears completely. In this fashion it comes about in this animal that only the left sixth arch is involved in the permanent pulmonary circulation (Figs. 317, 319). Meanwhile there develops within the truncus arteriosus a septum dividing it into two channels. One as usual leads from the left ventricle to the systemic aorta, and the other from the right ventricle to the single *pulmonary artery*. In the Bird it will be recalled that the portion of each sixth (pulmonary) aortic arch between it and the respective main aorta persists until hatching as a *duct of Botallo* or *ductus arteriosus*. In the Pig and other Mammals, however, only the left so persists. Its embryonic function and ultimate fate are similar in the Mammal to what they were in the Chick, and will be referred to again in connection with the development of the heart.

The Intersegmental Aortic Branches and Their Derivatives.

— It may be recalled that the Pig like the Chick has intersegmental arteries, and that anterior to the seventh cervical they have fused to form the vertebral and basilar arteries. It remains to note their further development as follows:

Posterior to the seventh cervical, the intersegmentals in the anterior part of the thorax also become fused antero-posteriorly, and disconnected from the aorta. Thus independent longitudinal vessels are produced in this region also (Fig. 317). Here, however, they come to supply the breasts, and are known as the *mammary arteries*. Returning now to the seventh cervical intersegmentals, it will be recalled that at 10 mm. these vessels have started to enlarge slightly in connection with the development of the subclavians. In fact the left one, continuing to enlarge, comes to constitute the entire left subclavian, which as noted, thus takes its permanent origin from the dorsal aorta. The right seventh cervical also enlarges, but only forms the distal part of the right subclavian. This is because the proximal part on this side is formed from the right fourth aortic arch, and a short portion of the right dorsal aorta

between the arch and the origin of the right seventh cervical. The part of the right dorsal aorta posterior to its junction with the seventh cervical of course disappears. Reference to figure 319 will make it clear how these developments result in the origin of both the vertebral and the mammary arteries on either side from the subclavians.

It is of some interest in connection with this origin of the subclavians to recall that in the Chick the so-called primary subclavians arise as branches of the eighteenth segmental arteries. Then a shift later occurs so that the permanent subclavians arise from the common carotids. In the Pig, as we have seen, it is the seventh cervical intersegmentals that are involved in the development of the subclavians, both originally and finally.

The Aorta and Its Branches Posterior to the Heart.—The origins of the coeliac and anterior mesenteric arteries have already been noted as occurring at 10 mm. The more anterior of these, the coeliac, eventually comes to supply the stomach, liver, pancreas and spleen, while the anterior mesenteric passes mainly to the anterior and middle intestine. Posterior to the anterior mesenteric the renal arteries grew from the aorta at 10 mm. in connection with the mesonephros. Eventually when the metanephros develops, other arteries in close association with the original mesonephric vessels supply the new organs. The *posterior* or *inferior mesenteric artery* had not arisen at the 10 mm. stage, but develops at about 12 mm., and sends branches to the posterior part of the intestine at approximately the point where the latter emerges from the body-stalk. It continues to supply this part of the alimentary tract.

The largest branches of the aorta during fetal life in the Mammal are the large umbilicals whose origin has already been mentioned. It was also noted that even at 10 mm. each of them had given rise to a small branch, the external iliacs. These increase in size as the hind limbs develop, and finally at birth they become the main arteries supplying the hind legs. At the same time parts of the former umbilicals within the body, but distal to the point of origin of the external iliacs, persist as small branches, the *internal iliacs*. The parts of the umbilicals proximal to the external and internal iliacs remain as the *common iliacs*.

THE VEINS

Derivatives of the Omphalomesenterics.—“By 10 mm. the yolk-sac had virtually disappeared, and with it the omphalomesenteric veins leading to it. However, as was noted, the parts of these vessels within

the body proper altered to produce the hepatic portal system. This consisted of the two hepatic veins, the liver capillaries, and a single hepatic portal vein, with branches draining blood from the intestine. This is essentially the adult situation.

The Umbilical Veins. — When last noted there were two of these within the body, though the right one was becoming smaller (Fig. 321). Presently this latter vessel disappears anteriorly, while its caudal part persists for a time as a small vein draining the body wall posteriorly into the left umbilical. The latter vein increases its size within the liver where, as noted, it forms the posterior major portion of the ductus venosus. Also, as this occurs, it comes to lie nearer the mid-line, and thus to pass between the two hepatic veins, which enter it at about the same point as the hepatic section of the developing posterior vena cava. As previously noted, the short anterior section of the ductus which empties into the sinus venosus, and was formed from the fused vitelline veins, now receives the hepatics, the major part of the ductus, and the hepatic portion of the posterior vena cava. Thus this short section becomes the anterior extremity of that vessel. Therefore since the anterior remains of the posterior cardinals empty into the ducts of Cuvier, it comes about that the posterior vena cava is the sole vein entering the sinus from the back part of the body. The further development of the posterior parts of this important vessel will be considered presently. As to the fate of the left umbilical, its function of course ceases entirely at birth, the anterior portion of its path (the ductus venosus) being marked by a fibrous strand, the *round ligament of the liver*.

The Anterior Cardinal System and Anterior Vena Cava. — As described at 10 mm. the anterior cardinal system consisted of the anterior cardinal veins and their capillaries, and the external jugulars which joined the cardinals just anterior to the ducts of Cuvier. It was also noted that each subclavian, consisting of an enlarged intersegmental vein, entered the posterior cardinal virtually at the point where anterior and posterior cardinals passed into the respective Cuvierian ducts (Fig. 322, *E*). Continuing with the subsequent story it may now be stated that with the caudal shift of the heart and ducts of Cuvier, these parts soon come to lie posterior to the limb buds. As a result of this the entrance of the subclavians shifts forward so that presently they definitely empty into the anterior cardinals (Fig. 322, *F*).

The next steps consist in the shifting of the previously symmetrically arranged veins so that they enter the right side of the heart. This is brought about mainly by the development of a diagonally transverse

vessel. This vessel runs from the junction of the left subclavian with the left anterior cardinal, across to the right anterior cardinal, slightly posterior to the point where that vessel receives the right subclavian. In the meantime the left anterior cardinal posterior to the origin of the new vessel disappears (Fig. 322, *H, I*). Hence all the blood from the left anterior region, along with that from the right, now has to enter the sinus venosus through the right anterior cardinal and duct of Cuvier. With these changes the vessels concerned have their adult arrangement, and may be given their adult names. The new transverse vessel is the *left innominate vein*. The section of the former anterior cardinal between the junction of the left innominate with this cardinal and the entrance of the right subclavian, is now the *right innominate vein* (Fig. 322, *J*). The posterior or proximal portion of the right anterior cardinal between the entrance of the left innominate and the right duct of Cuvier, plus that duct, is now the *anterior vena cava*. As will presently appear both posterior cardinals have by this time disappeared as such, though certain remnants persist which will be described below. Finally the distal parts of both anterior cardinals cephalad to the points of entrance of the respective subclavians and external jugulars are now termed the *internal jugulars*.

The Posterior Cardinal System, Posterior Vena Cava and Related Vessels. — It will be recalled that at about 10 mm. the posterior cardinals had practically disappeared at the mesonephric level. Their posterior remains, however, drained into the newly formed median anastomosis of the subcardinal sinuses through numerous capillaries. Anteriorly the left subcardinal had almost lost its connection with the anterior part of the left posterior cardinal. At the same time the right subcardinal had established a connection with the newly formed median vessel passing through the liver to the sinus venosus. This vessel, together with the subcardinal sinus and remains of the right subcardinal then constituted the anterior part of the posterior vena cava. Its establishment, as noted, has thus produced the essentials of a renal portal system. The final step in this process is the complete severance of the connection of the left subcardinal vein with the posterior cardinal which occurs very shortly after the 10 mm. stage (Figs. 320, 322, *C, D, E*). The further development of the posterior venous system then proceeds as follows:

The posterior parts of the posterior cardinals have from an early period received the external and internal iliac veins which form in connection with the posterior limb buds. These cardinals, however, are gradually replaced by a new pair of cardinals close to the dorsal body

wall, and hence called the *supracardinals* (Fig. 322, *F*). The external and internal iliacs then become attached to these new supracardinals (Fig. 322, *F*, *H*) through the stumps of the old posterior cardinals, now termed the *common iliacs*. In the region of the subcardinal sinus, (the present end of the posterior vena cava) the supracardinals become connected, at first through capillaries, and then by larger channels, with this sinus. Just anterior to this region the supracardinals are slightly developed and presently disappear, though still further forward they continue to exist and to connect with the anterior remains of the old posterior cardinals (Fig. 322, *I*). We shall return to this situation presently. Continuing with the account of the more caudal region, however, we find that the final steps here are: (1) the degeneration of the left supracardinal, (2) the connection of the left common iliac with the end of the right supracardinal, and (3) the shift of the latter to the median line. The result of this is to make the surviving supracardinal the posterior extension of the posterior vena cava, thus completing that vessel in its caudal extent (Fig. 322, *H*, *I*, *J*). Anteriorly the portion of it within the liver finally works its way to the dorsal surface where it becomes quite conspicuous before opening into the right atrium of the heart in a manner to be indicated presently.

Returning now to the more anterior parts of the supracardinals, and the remnants of the posterior cardinals into which they drain, we find that these vessels persist somewhat irregularly as the *azygos veins*. Generally the latter are united transversely, one or the other loses its anterior connection, and both drain into the anterior vena cava through the remains of a posterior cardinal, now termed the *cervico thoracic*, though in the Pig this may not occur (Fig. 322, *J*). Hence it may happen that the left duct of Cuvier is left with no (or in the Pig, few) tributaries. In any event it does not disappear, but instead becomes imbedded in the heart muscle as the *coronary sinus*.

In conclusion of this discussion it remains to state that while these changes have been going on both anteriorly and posteriorly the sinus venosus has been absorbed into the right atrium of the heart. Hence, since the sinus previously received the anterior and posterior vena cavae and the coronary sinus, this final change means that these three vessels ultimately open separately into the right atrium.

The Pulmonary Veins.—It will be recalled that at 10 mm. the pulmonary veins entered the left atrium of the heart by a common trunk. It now remains to state that eventually this trunk is incorporated into the atrium, and its two or more branches achieve separate openings.

The Heart. — When last described at 10 mm. this organ consisted of a ventro-posteriorly directed ventricle and antero-dorsally directed atrium. The walls of the former were lined by spongy tissue, the trabeculae carneae, and the chamber was partly divided by a septum growing toward the atrio-ventricular canal. In the latter the fusion of the

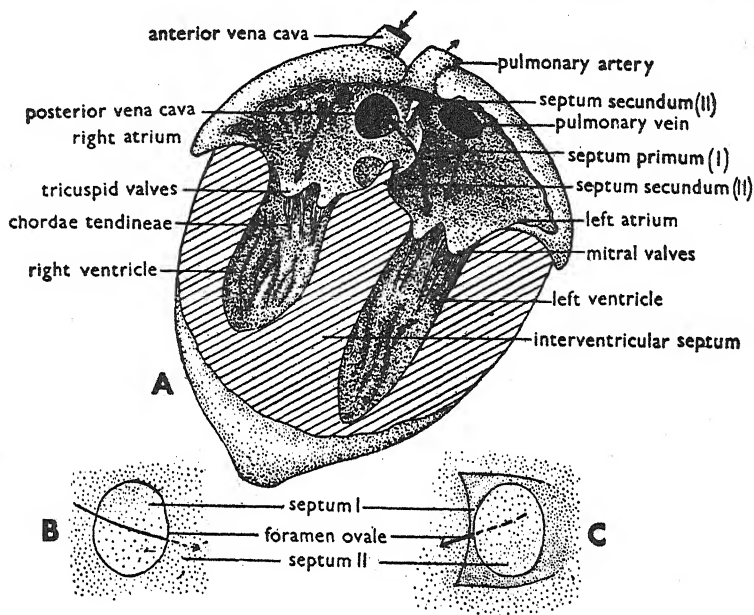


Fig. 336. — Drawing of fetal Pig heart at nearly full term, opened from the ventral side. *B*. Semi-diagrammatic view of the foramen ovale and septa I and II from the right side. *C*. Same from the left side. Arrows in all cases represent directions of blood flow according to the most recent conclusions. In *B* and *C* the dashed parts of the arrows indicate that a membrane lies between the arrow and the observer. For a complete discussion of the flow of blood in the embryo of the Chick and the Mammal see the text on this topic in the account of the Chick, and Fig. 236X.

cushion septa had almost, or quite, completed the division of this orifice into right and left channels. At the same time the atrium had been nearly divided by the septum primum growing from its antero-dorsal wall. As was indicated, however, this septum had already developed an opening in its antero-dorsal region called the interatrial foramen secundum. The right atrium received the sinus venosus, and the left the single pulmonary vein. Further development may now be described as follows:

The completion of the cushion septum if not accomplished at 10 mm.

soon takes place. This is then quickly followed by the completion of the interventricular septum, and also that of the interatrial septum primum. This latter event closes the interatrial foramen primum, but leaves wide open the recently developed interatrial foramen secundum. The heart therefore is now completely divided into right and left parts except for this latter opening. Meanwhile there has developed another atrial septum just to the right of the first, called the *septum secundum*, the beginning of which was shown at 10 mm. (Fig. 313). It too is a crescent-shaped sheet extending from the antero-dorsal wall of the atrium along its dorsal and ventral walls. Presently it extends all around these walls and fuses with the septum primum near the atrio-ventricular cushion septum. The new septum secundum, however, fails to become complete in its central region just ventral to the interatrial foramen secundum of the septum primum. This opening in the new septum is called simply the *foramen ovale*. As reference to Figure 336 will show its position is such that the middle part of the septum primum acts as a valve which can functionally close the foramen ovale. Such closure would obviously occur if pressure were applied to the valve from the left side. We shall return to this matter presently.

Meanwhile as the septa have been thus completed certain further events have taken place. On the sides of the atrio-ventricular canals flaps of tissue have developed, two on the left side and three on the right. These form the *atrio-ventricular valves* (tricuspid right, and mitral left) which hang downward into the respective ventricles. Here their edges have remained attached to some of the trabeculae carneae, which in these particular instances become drawn out into strands, the *chordae tendineae*, continuous ventrally with the *papillary muscles*. These, however, are not all the valves of the heart. As previously noted, the truncus arteriosus also becomes divided by a septum into two channels, the systemic and pulmonary, which lead respectively from the left and right ventricles. It now remains to state that at its union with the heart the truncus, previous to its division, develops upon its walls two thickenings. Then with the growth of the dividing septum these thickenings are transformed into six *semilunar valves*, three in each channel.

Finally in the atrial region it has already been remarked that the sinus venosus has been incorporated into the heart on the right side, and the single pulmonary trunk on the left. This of course causes the separate veins previously opening respectively into the sinus and pulmonary trunk to open directly into the right and left atria. In connection with this it remains to state that as this occurs portions of the right *valvula*

venosa of the sinus are retained as valves of the caval and coronary openings. Also in the later stages of development the atria of the Pig and other Mammals acquire the more or less earlike appendages which have given rise to the name *auricle*. These it may be recalled occur in the Bird, but only to a slight extent, and not at all in the Frog.

The Fetal and Adult Circulation. — This topic was discussed at considerable length in the case of the Chick, and since essentially the same situation is involved in the Mammal we shall not repeat it here. The student is urged to reread that section at this point. If this advice is followed it will be noted that the chief item of difference cited between the Bird and the Mammal concerned the character of the interatrial opening and its method of closure. There was only one septum in the Bird, corresponding to the mammalian septum primum, and instead of a single opening it contained several. These were closed at hatching by the equalization of pressure on the two sides of the septum which took the stretch out of it, and allowed the perforations to close by contraction. In the Mammal there is the same equalization of pressure at birth. In this case, however, the result is to press the valvelike part of the septum primum against the foramen ovale in the septum secundum, and thus functionally to close that opening. The actual fusion of the parts of the two septa does not occur for several weeks and sometimes several months post partum. Indeed a probe patency may exist permanently, but so long as equal pressure in the atria is maintained, this is of no consequence. The closure of the duct of Botallo was also noted in the discussion of this topic in the section on the Chick, and it was indicated that its permanent closure in the Mammal might occur in about a month. As a matter of fact the time varies in different animals, being 3–4 weeks in the Pig and 6–7 weeks in Man. The relation of the failure of the closure of the septum or of the duct to infantile cyanosis in Man was indicated in the discussion of this topic in the Chick (Figs. 236X, 336).

THE URINOGENITAL SYSTEM

THE EXCRETORY SYSTEM

The Mesonephros. — When the excretory system was last discussed the pronephros had entirely disappeared, and the mesonephros was well developed and functional. Indeed it is relatively larger at this and immediately subsequent stages than when it reaches its peak in absolute size and activity. Thus it continues to grow and function for some time

beyond the 60 mm. stage, when it is replaced by the metanephros. In the male of course certain parts of the mesonephros persist permanently in connection with the reproductive system as will be indicated presently.

The Metanephros. — The origin of the permanent kidney or metanephros has already been indicated. Thus at 10 mm. each of these organs consists of a short tubular outgrowth from the postero-dorsal side of the respective mesonephric duct just short of the point where the latter enters the cloaca. At its anterior end this outgrowth, the future ureter, has an enlargement, the anlage of the future pelvis of the kidney. Surrounding this is a concentration of nephrogenic mesoderm (Figs. 296, 323).

Further development consists in the forward growth of the ureter and its pelvic enlargement, which carries with it the nephrogenic mesoderm to a position dorso-lateral to the middle of the mesonephros. Meanwhile from the pelvic enlargement there have grown out into the surrounding nephrogenic substance numerous outgrowths which soon become hollow, and which represent the *collecting ducts*. At the same time concentrations within the nephrogenic mesoderm have become vesicular, and the vesicles send forth outgrowths which become tubular and connect with the collecting tubules. Later these outgrowing secreting tubules become even more convoluted than in the case of those of the mesonephros. Finally, each vesicle becomes invaginated by a *glomerulus*, and thus is transformed into a *Bowman's capsule*. The blood supply to both glomeruli and tubules is entirely arterial in the metanephros. This supply also differs from that to the mesonephros in that it is furnished to each permanent kidney by one main renal artery instead of by several smaller branches.

The details of development of the caudal outlets of the ureters and mesonephric ducts can best be described in connection with related parts of the reproductive systems, and will be taken up presently. Before proceeding to that topic, however, there remains a word to say about certain other organs closely connected with the kidneys, though not excretory.

The Adrenals. — As we have seen in the case of the Frog and Chick, these structures vary considerably in form, but always consist of two parts having specific origins. The medullary substance develops from cells which have their origin in the neural crests. These cells migrate from the crests along with some of the cells which are to form the sympathetic ganglia, and many of them, after acquiring a special staining capacity, become associated with these ganglia. Others, now called

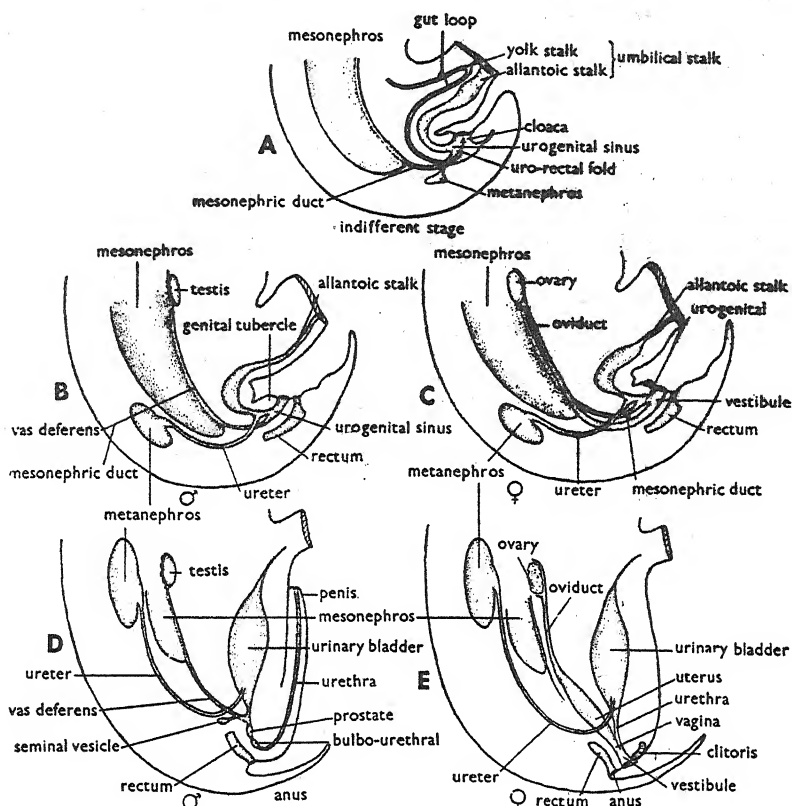


Fig. 337. — Semi-diagrammatic illustrations of the development of the metanephros, the adult ureters and gonoducts, and the separation of the cloaca into anal and urino-genital regions in the Fig. A. Unseparated cloaca with no indication of sex differentiation (about a 10 mm. embryo). B and D. Progressive separations of the cloaca and development of the urino-genital ducts of the male. C and E. The same process in the female.

chromaffin cells, come to lie beneath the mesoderm of the coelom. The larger number of these chromaffin cells, however, form a mass adjacent to the cephalic end of the kidneys, where they form the adrenal *medulla*. Around this medullary substance which becomes arranged in cords, there then accumulate mesodermal cells which constitute the adrenal *cortex*.

THE REPRODUCTIVE SYSTEM

The Gonads. — The later development of both testes and ovaries has been previously described at some length in general and in connec-

646 THE LATER DEVELOPMENT OF THE PIG

tion with specific forms. It is essentially similar in all these cases, except in regard to certain aspects of the mammalian ovary, which were also considered previously when mammalian oögenesis was discussed.

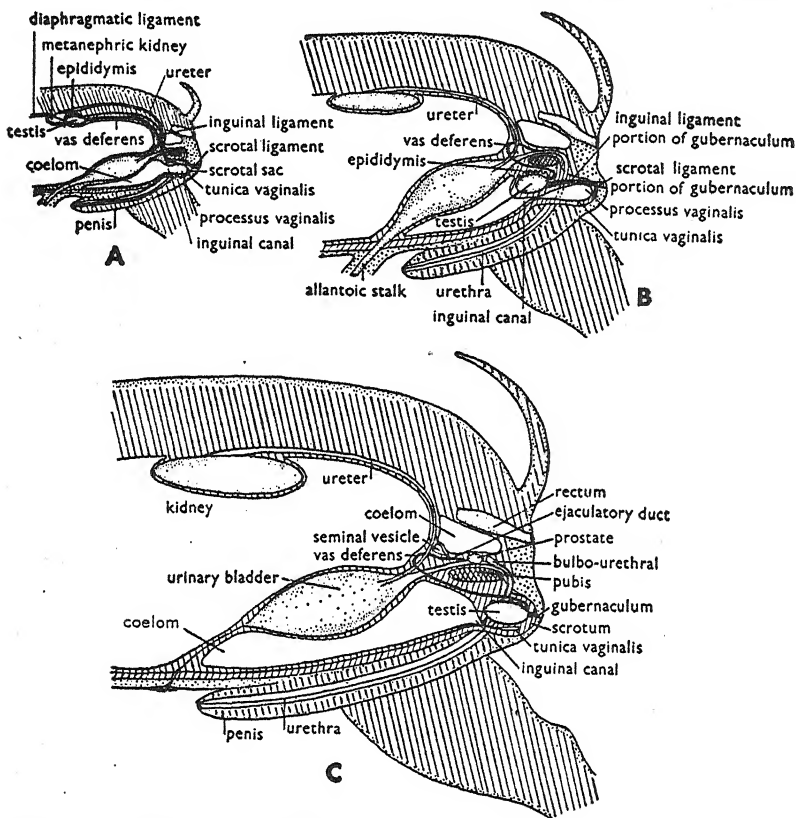


Fig. 338. — Diagrams representing the descent of a Pig testis. *A.* Before the testis has started to move. *B.* The testis about to enter the scrotum. *C.* The testis in the scrotum.

We shall not therefore go into this subject again in connection with the Pig.

The Male Urinogenital Ducts. — As we have seen in the case of the Bird, so in the Mammal, the mesonephric duct when no longer needed as a ureter is pressed into service as a sperm duct, or *vas deferens*. Anteriorly the connection between this duct and the respective testis is made through certain mesonephric tubules which are retained for this purpose. They, together with the immediately adjacent portion of the

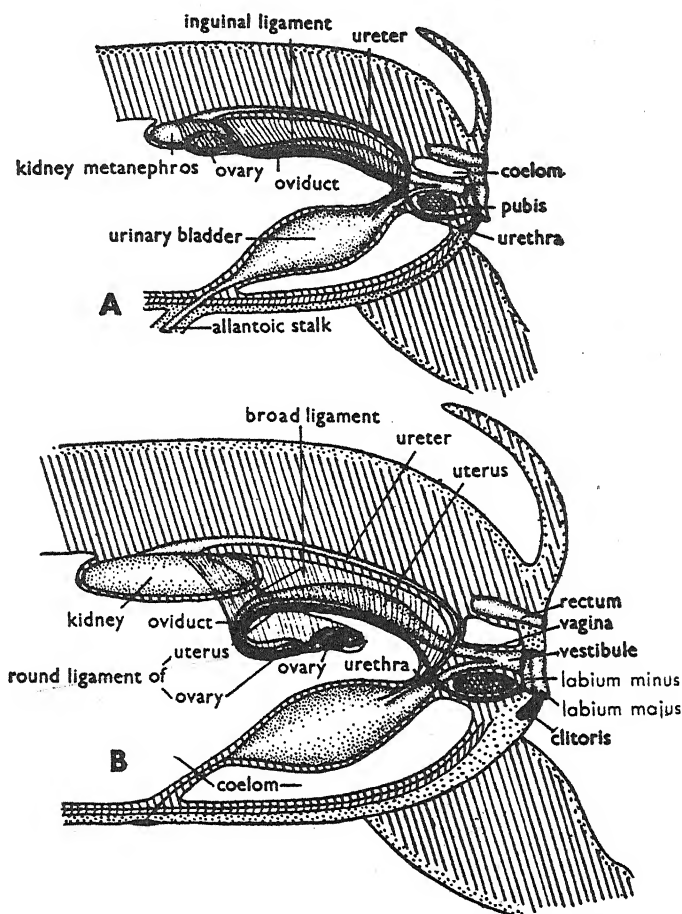


Fig. 339. — Diagrams representing the partial descent of a Pig ovary. *A.* Before the ovary has started to move. *B.* After it has reached its definitive position.

mesonephric duct become the *epididymis*. The extreme anterior remnant of the mesonephros may persist as the *appendix to the epididymis*, while the vestigial caudal remainder occurs as the *paradidymis*.

At its caudal end the mesonephric duct when last noted was emptying into the antero-ventral part of the cloaca, which was being separated off as the urinogenital sinus. This division of the cloaca into urinogenital and rectal portions by the urorectal fold is presently completed, and shortly thereafter the cloacal membrane is ruptured. This of course

puts both cloacal parts in communication with the proctodaeum, the opening of the urinogenital sinus being termed the *ostium urogenitale*, and that of the rectum, the *anus* (Figs. 337, 340). At the same time that this has been going on the part of the allantois inside the body has been dilating to form the *urinary bladder*. Presently when the urinogenital sinus, into which the allantois opens, becomes completely separated from the rectum, the cephalic part of the sinus also expands somewhat. Thus this part is in effect simply added to the posterior end of the bladder, forming its proximal portion. The more caudal portion of the sinus, however, is narrowed instead of dilated, and becomes the *urethra*. While this has been taking place the end of the mesonephric duct into which the metanephric duct opened has been drawn into the urinogenital sinus, so that these ducts now open separately. Furthermore, the cephalic growth of the metanephros seems to have pulled its duct forward somewhat. The result is that when the separate openings are achieved, that of the metanephric duct is into the antero-lateral part of the old urinogenital sinus, now forming the base of the bladder. The opening of the old mesonephric duct, however, now the vas deferens, is further posterior into the part of the sinus which now forms the urethra (Figs. 337, 338).

It remains to add that slightly anterior to the point where the vas deferentia enter the urethra each becomes dilated, and the dilation drawn out slightly to form a small sac, the *seminal vesicle*. The short remaining part of the vas deferens between the vesicle and its entrance into the urethra is termed the *ejaculatory duct*. Finally the urethral epithelium gives rise to two glands on the outside of the urethral lumen, but with openings into it, the *prostate* and the *bulbo-urethral* or *Cowper's gland* (Figs. 337, 338). This concludes the part of the male urinogenital duct system which is, so to speak, within the body. The remaining portion, together with a description of the ultimate disposition of the testes, will be taken up presently. Before doing that, however, we must return for a moment to the development of the ducts of the female, and certain other considerations.

The Female Urinogenital Ducts. — The *oviduct* originates in the Mammal, as it has been seen to in the Frog and Chick, from a thickened ridge of mesoderm lying along each side of the mesonephric duct. This ridge becomes tubular and pulls away from the body wall, to which it remains attached by a fold of peritoneum supporting both ovary and duct. This fold or double sheet of tissue, homologue of the Chick mesovarium, is called the *broad ligament*, of which more will be said later (Fig. 339). There are of course two oviducts, one on either side, and they

at first open separately into the urinogenital sinus. Very shortly, however, their caudal ends fuse to form the *vagina*. Anterior to this each duct becomes differentiated histologically into a part called the *uterus*, and still further forward into the definitive oviduct or *Fallopian tube*. As has already been indicated in our introductory discussion of the Mammal, the degree to which the uterine portions of each duct later fuse to form a single uterus varies in different kinds of animals. In all but the most primitive, however, a slight fusion always occurs to form a region known as the *cervix* opening into the vagina by a single orifice. In the Sow and other Ungulates this fusion continues a short distance anterior to the cervix to produce a typical uterus bicornis; in Man, of course, the fusion of the uterine parts is complete, giving a uterus simplex. At their anterior ends each oviduct, as has been seen, develops a funnel or infundibulum which may or may not embrace the ovary. In the Sow it does, but in Man it does not. In any event it is of interest to find that this anterior opening develops, not quite at the anterior tip of the original tube, but slightly caudal to it.

So far as the excretory ducts of the female are concerned the ureter comes to open into the base of the bladder following the division of the cloaca, just as it does in the male. The mesonephric duct naturally has no function in the female, but does persist, along with parts of the mesonephros as a vestige. There are as a matter of fact several of these vestiges in both sexes in addition to those already indicated. Some of these are outside the body, and will be referred to later. Confining ourselves for the moment, however, to those within, it will be well at this point to make some further reference to these remnants.

Internal Vestiges of the Reproductive Systems. — The vestigial appendix of the epididymis and the paradidymis respectively have already been noted. In addition to these in the male, a vestige of the oviduct may be found in the tissue investing the testis, where it is called the *appendix of the testis*. Posteriorly also a further vestige of the fused parts of the oviducts may occur as the *uterus masculinus*. In the female the undifferentiated anterior tip of the oviduct often remains as a small vesicle attached to the duct. Also a vestige of the mesonephros is usually embedded in the broad ligament (mesovarium) as the *epoöphoron*, a structure previously mentioned as occurring in the Chick. Finally vestiges of the mesonephric duct, or parts of it, may remain near the uterus and vagina as the *canals of Gärtner*.

We are now prepared to return to a consideration of the migration of the gonads, and to the development of external features connected with

both male and female systems. We shall consider the movement of the gonads first, and we shall begin with the testes.

The Descent of the Testes. — The student is well aware of course that in the lower animals, such as the Fishes, Amphibia and Reptiles the testes remain within the body at their places of origin. Indeed this is even true in the Birds, which in their way are quite as "high" or specialized as the Mammals. It is only within the latter group, however, that the testes radically alter their position so that in most cases they are actually outside the original body cavity all or part of the time. How this comes about is now to be considered.

Both the mesonephros and adjacent testes are held against the body wall by a covering of peritoneum. As they grow they push this covering out into the coelom, but the covering does not cut in above them to form a mesentery-like sheet. Instead they simply remain beneath it, such a position being described as retroperitoneal. As development goes on the testis becomes relatively larger and the mesonephros relatively, and finally absolutely smaller, so that the former occupies more and more of the retroperitoneal space. Meanwhile, though the peritoneum (mesodermal epithelium plus connective tissue) does not cut in above the testis and mesonephros, anterior and posterior to them it is drawn out into a longitudinal fold within whose layers runs a bundle of connective tissue fibers. Anteriorly the fold and its bundle of fibers extends from the mesonephros to the diaphragm, and is known as the *diaphragmatic ligament* (Fig. 338, *A*). The posterior section of the fold and fibers reaches to the extreme caudal end of the coelom, this section being termed the *inguinal ligament of the mesonephros*. Here a pair of coelomic evaginations occur, the *scrotal sacs* or *pouches*, the cavity in each being termed the *processus vaginalis*. From the distal wall of each pouch a fibrous strand, the *scrotal ligament*, proceeds beneath the epithelium to the coelom proper. There each scrotal ligament becomes united to the caudal end of the respective inguinal ligament of the mesonephros (Fig. 338). Here it should be incidentally noted that this inguinal ligament has nothing at all to do in origin or function with the inguinal ligament of the adult, known in Man as Poupart's ligament.

While this is occurring posteriorly the testis is outstripping the mesonephros in growth, and as it does so the attachments of the diaphragmatic and inguinal ligaments of the latter organ become transferred to the former. When this has taken place the united inguinal and scrotal ligaments are given a single name, the *gubernaculum*. Thus it comes about that a gubernaculum extends from the caudal end of each testis

and adjacent epididymis to the bottom of each scrotal sac. We might now briefly complete the story by simply saying that while the diaphragmatic ligament stretches the gubernaculum contracts, thus pulling the testis and epididymis back and down into the scrotal sac. Essentially this is what happens, but as a matter of fact the gubernaculum does not contract. It merely fails to grow, while the other parts do, so that the effect is the same as if it did contract. (It is like the case of the boy holding the cat's tail. He does not pull it. The cat does that.) In the course of this movement the vas deferens is bent into a loop which passes across the permanent ureter.

It must now be pointed out that since the testis is retroperitoneal it does not actually lie in the coelomic space of the scrotal pouch (processus vaginalis) any more than it lay in the general body coelom. Instead it is pulled down all the way beneath the peritoneal covering which within the pouch is reflected over it as the *tunica vaginalis*. Of course in this process the coelomic space within the scrotal sac is eliminated. While this space existed, however, it was connected with the general coelom by the *inguinal canal*. From what has just been said it must also be clear that the testes do not really pass into the pouches through the canals, though the existence of the canals permits the movement. They pass back of the canals underneath the peritoneum. After the testes have thus gone into the scrotal sacs the inguinal canals fuse completely shut, except in a few animals to be indicated presently. Nevertheless, it is of interest that this spot evidently comprises a point of weakness which accounts for the occurrence of inguinal hernia in Man. The fact that it occurs in this case, but seldom if at all in the lower animals is probably the result of Man's erect position. There seem still to be certain advantages in walking on all fours.

It remains to state that the movement of the testes just described does not occur in all Mammals. Thus in the Elephant the testes remain permanently within the body, while in the Rat they pass back and forth, descending during sexual activity. In this connection it is significant that the temperature of the scrotum has been shown to be lower than that of the body cavity. Furthermore, experiment has proven that in animals in which the testes normally remain permanently in the scrotum the retention of the testes within the body results in sterility. Lastly, if in such animals the temperature of the scrotum is artificially raised to that of the body, sterility also results. Thus it appears that in these cases the temperature conducive to spermatogenesis and (or) sperm survival is lower than the normal body temperature.

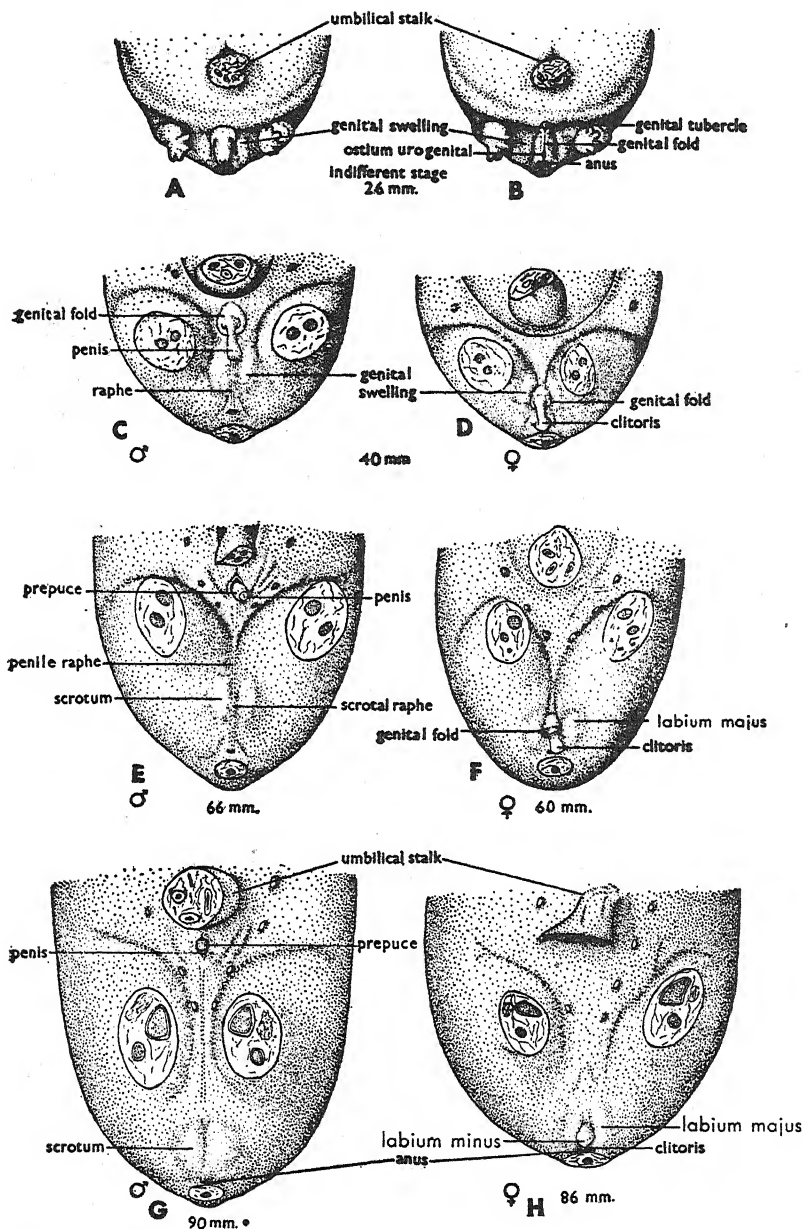


Fig. 340. — Drawings of stages in the development of the Pig external genitalia. *A* and *B*. The same indifferent stage preceding sexual differentiation. In *A* the genital tubercle and related parts are turned posteriorly. In *B* these parts are reflected anteriorly to show their ventral aspects. *C*, *E* and *G* represent the progressive development of the genitalia of the male at the stages indicated, while *D*, *F* and *H* represent corresponding development in the female.

The Descent of the Ovary. — In the case of the ovary and oviduct we have noted that these organs are attached to the coelomic wall by a fold of mesothelium and connective tissue called in the Mammal the broad ligament. Within this fold is enclosed the fibrous inguinal ligament of the mesonephros along with the vestiges of the epididymis (epoöphoron) and vas deferens (canals of Gärtner). In this instance as development proceeds the inguinal ligament (anterior part of the gubernaculum of the male) apparently exerts no traction. Rather the ovary and oviduct, pulled downward by their weight, stretch both the broad ligament and inguinal ligament within it. Shortly the ovary has moved so far posteriorly that both the oviduct and the ligament are bent around at a considerable angle. When this has occurred the part of the inguinal ligament between the ovary and the bend is called the *round ligament of the ovary*, and that part between the bend and the uterus the *round ligament of the uterus*. In this manner the ovaries come to lie much further back in the body than their point of origin, but unlike the testes they never pass outside (Fig. 339).

The External Genitalia, Indifferent Stage. — As in the case of the very early stages of the gonads themselves so also in this case an indifferent stage exists during which sex is indistinguishable. Also, as will presently appear, we find that the same fundamental structures occur in both sexes. It is only with later development beyond the 25 mm. stage that they begin to become differentiated to form the external urogenital parts of the adult male and female. The parts concerned and their locations are as follows:

As the urorectal fold is dividing the cloaca into the urinogenital sinus and the rectum, the proctodaeum surrounding the original common orifice essentially disappears as such (Fig. 337). Thus the orifice of the urinogenital sinus (the ostium urogenitale), the edge of the urorectal fold (the rudiment of the *perineum*) and the anus are brought virtually to the surface in this region. Just anterior to the ostium urogenitale there meanwhile appears a slight elevation known as the *genital eminence*, which shortly becomes more prominent, and is then called the *genital tubercle*. Immediately on either side of this tubercle lie a pair of folds called the *genital folds*. These folds lie not only at the sides of the tubercle, but also extend caudad enough to flank the ostium urogenitale causing the latter to become slit-like. Somewhat further to either side of the genital folds are another pair of elevations, the *genital swellings* (Fig. 340, A, B).

The External Genitalia, Male. — The genital tubercle becomes elongated, and grows forward to form the *penis*. The genital folds from

either side then grow around the penis to form the *prepuce*, while more posteriorly and to the sides the genital swellings are pushed out by the coelomic evaginations to form the coverings of the scrotal sacs. These presently fuse in the mid-line to produce the single *scrotum*, the line of fusion constituting a ridge called the *scrotal raphe*. Up to this point it will be noted that the penis lacks a canal. This is formed by a groove developing along its ventral side, the edges of which soon fuse, and thus is formed the *penile urethra*, extending from the tip of the penis to the urinogenital sinus. The part of this sinus between this point and the bladder then comprises the *prostatic urethra*. The line of fusion of the edges of the ostium urogenitale and those of the groove along the ventral or caudal side of the penis forms an extension of the scrotal raphe called the *penile raphe* (Fig. 340, C, E, G).

The External Genitalia, Female. — The situation in the female is considerably simpler. Starting from the same structures in the indifferent stage we find the tubercle forming a vestigial part at the anterior border of the ostium urogenitale. It is called the *clitoris*, and is obviously the homologue of the male penis. The urinogenital sinus itself becomes the *vestibule* which leads into the vagina formed from the fused ends of the uteri. Upon either side the ostium urogenitale of the vestibule is flanked by the genital folds which have become the *labia minora*, and slightly more laterally by the genital swellings which have become the *labia majora*. The former are of course the homologues of the male prepuce and the latter of the scrotal sac coverings. The term *vulva* includes all the parts just mentioned (Fig. 340, D, F, H).



THE SKELETON, TEETH, HAIR, HOOFS AND HORNS

THE SKELETON

IT is not the intention to undertake for the Pig, any more than we have done for previous forms, a detailed description of skeletal development. It does seem worthwhile, however, to point out a few of the outstanding similarities and differences in this development as it occurs in this animal and in the Frog and Chick.

The Skull. — As in the case of the Frog and Chick the bones of the Pig skeleton may be divided into membrane or dermal bones and cartilaginous bones. On this basis we find in the cranial part of the skull of this animal the same embryonic cartilaginous foundation which we have previously noted, i.e., the *basilar plate* (fused parachordals and notochord) and the *trabeculae*. Later of course these develop ossification centers giving rise to the *ethmoid* and certain of the *sphenoid bones*. Also added to the cranium from cartilage are the *occipitals* and the various bones forming the otic and nasal capsules such respectively as the *periotics* and the *naso-turbinals*. It will be recalled, however, that the primitive cartilaginous element of the upper jaw, the palato quadrate, still represented in the Bird by the quadrate, has in the Mammal apparently moved into the middle ear as the *incus*. Likewise in the lower jaw a portion of Meckel's cartilage, in the Mammal is thought to constitute the *malleus*. All the dermal bones, i.e., those ossifying directly from membrane which occurred in the Bird, exist also in the Pig, with the exception of the quadrato-jugals and parasphenoids. In the lower jaw dermal elements replacing the main remnants of Meckel's cartilage become ossified and fused together to form the single mandible.

The Vertebrae, Ribs and Sternum. — The concentrations of mesenchyme which are to form the *vertebrae* alternate with the original somites just as they did in the Frog and Chick, and surround the notochord. Cartilage forming centers then develop, one about the remains of the notochord, i.e., the future centrum, one in each neural arch and one in each costal process. The cartilage soon spreads from these centers to form a continuous cartilaginous structure for each future vertebra. Then ossification begins in the same centers where cartilage forma-

tion began, and spreads until each vertebra consists entirely of bone. The *rib* cartilage is at first continuous with that of the costal processes, but when ossification begins, the cartilage of the ribs becomes separated from that of the vertebrae, and each rib has its own ossification center. It is of interest that in correlation with the adult condition the cartilage in each rib of the Pig consists of a single piece, instead of two as in some of the ribs of the Bird. Although the cartilage of each rib is in this case in a single piece, this cartilage ultimately contains more than one ossification center. Thus the ribs in the Pig and other Mammals are like the long bones of the appendages in this class, in that the ends ossify separately from the shafts, forming the so-called epiphyses. As in the Bird the *sternum* has two cartilage centers attached to the rib cartilage on either side. Later these fuse in the median line.

The Appendicular Skeleton. — Considering the *fore limbs* first, we find the Pig shoulder girdle differing from that of the Bird in lacking both clavicle and coracoid. The only member of the girdle bones it does possess is the *scapula*, and this of course is a bone ossified from cartilage.

As regards the long bones of the fore limb (*humerus*, *radius* and *ulna*) we find that in the Mammal the method of ossification in all such bones differs somewhat from that in either the Frog or the Chick. Development begins as usual by the differentiation of cartilage from membrane. Around the middle (diaphyseal region) of this cartilaginous core the former perichondrium, now periosteum, starts to erode the cartilage and to deposit a band of bone. Since this band is soon thicker at its middle than at its ends, the remaining central cartilage presently becomes hour-glass shaped. Almost simultaneous with this outer deposit by the periosteum, the cartilage in the middle of the diaphyseal core also begins to be eroded by invading chondrioblasts, and its place is taken by bone deposited by osteoblasts. Soon this endochondral bone and that produced peripherally by the periosteum meet, and the diaphysis is entirely ossified. This bone, however, is all cancellous, and within it three changes occur. First, in the central axis of the diaphysis or shaft the bone is shortly removed and replaced by marrow. Second, about the periphery the original cancellous bone of both central and periosteal origin is also constantly removed and replaced as the diaphysis grows in diameter. Finally, as growth is completed the inner cancellous bone remaining at that time is remade by processes previously described, into compact Haversian systems. Likewise the outer cancellous periosteal bone is replaced by layers of compact periosteal bone. On the basis of

this description it might be questioned whether any of the ultimate diaphyseal bone is really endochondral, and it would appear probable that at least what occurs near the mid-region of the diaphysis is not. Nearer the ends, however, the case is different, and for the same reason that this was true in the Chick, i.e., because of the method of longitudinal growth. This method, though fundamentally similar to that in the Bird, differs in certain significant details, and is as follows:

While the processes described above are occurring toward the mid-region of the diaphysis each cartilaginous epiphysis is also undergoing ossification in one and sometimes two centers. In this manner there is presently produced in it a single disc of cancellous endochondral bone. At either end of the diaphysis, however, between the bone earlier formed in that location and the respective epiphyseal bony disc, there persists during growth a plate of cartilage known as the *epiphyseal plate*. These plates correspond in function to the cartilaginous ends of the growing bones of the Chick, i.e., they continue to produce cartilage distally and endochondral bone proximally on the side of each adjacent to the marrow cavity of the diaphysis. Finally, when growth ceases, the epiphyseal plate becomes entirely ossified, and thus joins the already formed bony epiphyses to the ends of the diaphysis. Hence it comes about that, as in the Bird, all of every epiphysis is endochondral. Also somewhat more of the mammalian diaphysis is endochondral because not so much of its interior is ultimately removed as is true in the Bird. For further details of bone histogenesis the reader is referred to the account of this process under the Frog, and to the accompanying figures.

The behavior of the digits has already been referred to in the Pig and we have noted that, as in the Bird, five digits are present in membrane. In the Pig, of course, the third and fourth are well developed while the first disappears and the second and fifth remain vestigial. The ossification of the metacarpals and phalanges occurs in these cases from cartilage in the same manner as in other mammalian long bones.

Posteriorly the *pelvic girdle* is ossified from three cartilages representing the *ilium*, *ischium* and *pubis*. As in the Bird they extend respectively anteriorly, posteriorly and antero-ventrally. In the Pig, however, the antero-ventrally extending pubic cartilages remain in this position, instead of rotating caudad to lie parallel with the ischia, as in the Chick. Thus when ossification occurs the pubic bones meet one another in the median ventral line, and are held firmly together by ligaments in the manner characteristic of Mammals. The long bones of the Pig *hind limb* are ossified in the same way as the long bones of the fore limb,

and consist of course of the *femur*, *tibia* and *fibula*. The four digits, two vestigial, are also formed as in the anterior appendages.

THE TEETH

As previously noted, although the Frog does develop teeth, they are small and late in forming so that nothing was said about them, while modern Birds have no teeth at all. It therefore seemed best to postpone

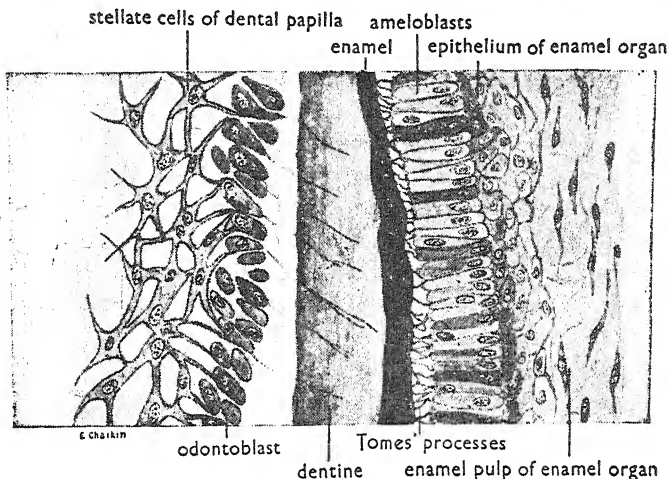


Fig. 341. — A sagittal section through a developing tooth, showing the cells responsible for the secretion of enamel and dentine, and the relations of these cells to those products.

an account of the origin of these structures until we came to the Mammal in which class they attain their fullest development. We shall not attempt to describe the development of any particular tooth since what is true for one is true for all in forms like the Pig or Man, save for variations in shape.

The Enamel Organs. — As has been previously indicated, at 30 mm. or shortly thereafter the originally single epithelial thickening termed the labio-dental ledge, has divided into two parts. The outer part presently forms the labio-gingival lamina or groove, and the inner one the dental ledge (Fig. 329). This ledge runs along the surface of an elevation which represents the *gum*, and at intervals along it the formation of the teeth occurs as follows:

At each point in the gum region where a tooth is to develop, there occurs a special ingrowth from the dental ledge which penetrates further into the mesenchyme than the non-tooth-forming part of the ledge. The

lower part of this ingrowth is expanded into a double-walled inverted cup, known as the *enamel organ*, which remains connected with the dental ledge for a time by a fairly stout neck (Fig. 329). The ledge in turn is also temporarily connected with the oral epithelium by a considerably narrower neck. The cells on the inner wall of the cup are columnar in shape, and are destined to secrete the enamel of the tooth. Hence they are called *ameloblasts*. Those in the outer wall are at first polyhedral, but soon become flattened, and are known as the *epithelium of the enamel organ*. The rather extensive space between the inner and outer walls of the cup is filled with a loose reticulate tissue termed the *enamel pulp*. Though all enamel organs start out with the relatively simple cap shape that has been indicated, each later assumes the contours characteristic of the crown of the tooth whose enamel it is to form (Figs. 329, 341).

The Dental Papilla. — As the enamel organ pushes into the mesenchyme the latter necessarily comes to occupy the cup which the organ forms, by which process this mesenchyme comes to constitute the *dental papilla*. Of course where the tooth is to have several cusps and roots the enamel organ develops more than one cup, and therefore gives rise to more than one dental papilla and parts subsequently related to it. Presently through multiplication the cells constituting the bulk of a papilla form a rather dense aggregation. At the same time those at its surface adjacent to the ameloblasts of the enamel organ become columnar like the ameloblasts. These columnar cells of the papilla are then ready for the secretion of their special product, the *dentine*, and are termed *odontoblasts*. It thus presently comes about that while the ameloblasts of the enamel organ secrete enamel to form the surface of the tooth, the odontoblasts secrete dentine beneath and adjacent to the enamel. As this activity begins to get under way the enamel pulp lying between the outer epithelium of the enamel organ and its ameloblasts, largely disappears, thus placing these two layers almost in contact. Probably this is significant in bringing the now active ameloblasts that much closer to their external blood supply. At the same time nerves and blood vessels penetrate the central tissue of the dental papilla, which gradually becomes transformed into the *pulp cavity* of the completed tooth. By the time these processes are under way, the enamel organ has lost all connection with the dental ledge.

Formation of Dentine. — The formation of the dentine by the odontoblasts is in some respects similar to the formation of circumferential bone by periosteum. In both cases it involves the deposition of

calcium salts about organic fibers (ossein fibers). In the case of the dentine, however, the product is not laminated, i.e., in layers, but is continuous. Also no cells are left entrapped within the calcareous substance, and the organic material is less abundant, about 28 percent in dentine as compared with 45 percent in bone. Hence the dentine is harder even than compact bone. Otherwise the materials are similar in that the calcium salts are permeated with ossein fibers, both fibers and salts being produced by the odontoblasts. Likewise there are processes of the odontoblasts which extend into the hard matrix just as the living processes of osteoblasts extend into bone. In this instance, however, the processes all come from the layer of odontoblasts at the inner surface of the dentine, since none are embedded within it, and they are known as the *fibers of Tomes* (shown but not labeled in Fig. 341). They are in general at right angles to the secreted ossein fibers. Obviously the continued production of dentine forces the odontoblasts away from the enamel, and also reduces the size of the original pulp cavity, until it becomes not much more than a canal. This canal continues to contain blood vessels and nerve fibers in intimate contact with the odontoblast layer which ultimately becomes inactive and simply lines the *pulp canal*. Since these inactive odontoblasts send the living fibers of Tomes clear through the dentine, it is easy to understand why this substance is sensitive when injured by decay or bored into by a dental drill.

The Formation of Enamel.—The enamel, as already indicated, is produced by the ameloblasts of the enamel organ. Because of the relation of these cells to the odontoblasts, moreover, the layer of enamel will necessarily lie adjacent to, and on the outside of, the dentine, or rather a part of it. As will shortly appear, and as reference to Figure 329 will show, the enamel organ, and hence the enamel, only covers the future crown of the tooth, not its roots. These are covered by other material whose origin will be described presently. In the region of the crown where the ameloblasts are at work we find that the layer they produce consists of microscopic prisms of very hard calcium salt crystals called *dahlite*. These are held together by small amounts of a different substance called cement. It seems to be clear that each prism of the enamel is produced by a single ameloblast, and therefore extends all the way from one side of the layer to the other. Since the prisms are not straight, or precisely parallel to one another, however, this is difficult to demonstrate in section. Organic matter is present, but in even smaller amounts than in the dentine, about 5 percent of the total substance being so constituted. It apparently consists mainly of fine protoplasmic processes

from the ameloblasts which are often called the *processes of Tomes* (Fig. 341). They evidently correspond to the similarly named processes or fibers put out into the dentine by the odontoblasts. Finally it is obvious that as the tooth grows outward due to the formation of more dentine underneath, the crown will presently be forced through the surface of the gum with the concomitant destruction of the enamel organ. When this has occurred it is evident that no more enamel can ever be formed, and that what has formed will extend only to the gum line. Hence if this hard covering of the exposed surface is later destroyed in any way it is gone forever. Dentine, on the other hand can be, and often is, added to from within, if in later life some of it is removed, as is the case when a tooth is filled. From what has just been said it also follows that unlike the processes of Tomes in the dentine, those of the enamel must disappear when the ameloblasts cease to exist.

The Formation of Cementum. — It has already been noted that only the crown of the tooth is covered by enamel, and that a different material covers the dentine of the root. This material is called *cementum*, and is produced by the mesenchyme which surrounds the entire tooth and enamel organ previous to eruption. This mesenchyme is said to constitute the *dental sac* (Fig. 329). It is only in the neighborhood of the root, however, that the tissue of the sac produces cementum. Here its cells behave almost exactly like the osteoblasts of any periosteum, and the cementum with which they cover the root is essentially the same as periosteal bone. Indeed on its outer side where the cells of the sac are in contact with the jaw bone instead of the teeth, they do in fact add to that bone in the manner of any periosteum. As will be recalled ossein fibers are produced by the cells of such periosteum, and such is the case here, both on the side of the jaw bone, and on that of the cementum. It thus comes about that these fibers actually extend out of the cementum right into the bone of the jaw. In this manner therefore the tooth is very firmly anchored in its socket.

The Permanent Teeth. — Thus far no mention has been made of more than one type of dentition. As everyone is aware, however, the first set of so-called *milk teeth* is later replaced by the *permanent teeth*. This process, however, need not detain us long. The enamel organ for each second or permanent tooth arises from the dental ledge near that of the milk tooth. When the ledge disappears, the organ in question lies in a depression of the alveolar socket on the lingual side of the growing milk tooth, but develops no further at this time. Later this "tooth germ" goes through the same processes as occurred in the case of the

milk tooth. Meantime the root of the latter is absorbed, and the crown is pushed off by the growing permanent tooth beneath it.

Teeth with Open Roots.— It is of some interest to note that in some animals, notably the Rodents, the incisor teeth continue to grow throughout life. This is made possible by the persistence of a wide root canal and the constant addition of more dentine. To compensate for this the outer end of these teeth is continually worn down by the gnaw-

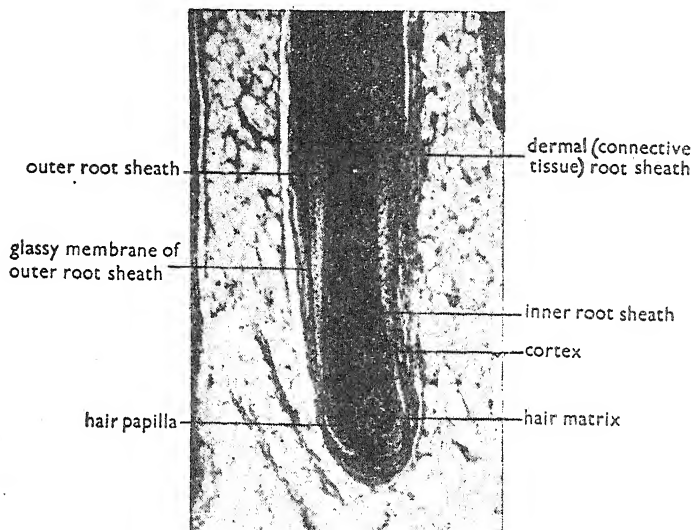


Fig. 342.—Photomicrograph of a mid-sagittal section through a hair root and papilla under high magnification.

ing activities of these animals. This furthermore is made possible by the fact that only the front side of the tooth is covered with enamel. The back side is dentine. Hence since enamel is much harder than dentine the wear is uneven, which gives the end of the tooth a constantly renewed chisel edge. Of course this process makes a continuance of enamel formation also necessary on the front surface of the teeth by the permanent existence of ameloblasts within the gum in this region, not found in other cases.

HAIR

Since hair develops long before the Mammal is born, and is one of the most characteristic features of the class, occurring nowhere else, it seems appropriate to refer at least briefly to its development.

As previously noted, hair like feathers is an epidermal structure, and again it actually consists of cells, not of a secretion by them like teeth. In this case the cellular character of hair is evident if it is examined under the microscope. Under these conditions its surface (*cuticle*) reveals transverse rows of wavy lines, which represent the edges of flat cells which overlap one another like the shingles of a roof. Beneath this cuticle are cornified layers of spindle shaped cells and their products, including pigment, which are termed the *cortex* (Fig. 342). In many types of hair, including that on the human head, the cuticle and the cortex constitute the entire substance of the shaft. In others, e.g., those of the beard, there is a restricted central region, the *medulla*, occupied by a few shrunken cells and numerous air spaces. The latter give such hairs a more silvery appearance when the pigment disappears with age. The base of each completed hair is contained in a tubular invagination of the epidermis. This invagination is called the *hair follicle*, and all of the parts which lie beneath the surface of the skin together comprise the *root*. The walls of this follicle consist of modified cells of the Malpighian layer of the epidermis, those next to the dermis constituting the *outer root sheath*, and those next to the hair the *inner root sheath*. The latter is itself usually divided into three separate cell layers, but these need not concern us here. At the base of the root these sheaths merge into dividing cells which are producing the substance of the hair, and pushing it upward through the lumen of the follicle. This mass of dividing cells is itself invaginated by an up-pushing bulblike portion of the dermis containing a blood vessel and known as the *hair papilla*. It is quite similar to the dermal invagination at the base of a feather called the feather pulp, and the function in both cases is to nourish the growing structure (Fig. 342).

Again, as in the case of the feather, the hair originates as a down-growth of the Malpighian layer termed the *hair germ*. A small upgrowth of the dermis invaginates the base or proximal part of this hair germ and constitutes the beginning of the hair papilla. Presently the central cells of the germ distal to the base become cornified and thus form the hair. The more peripheral cells of the distal part of the germ soon differentiate into the inner and outer root sheaths of the follicle indicated above. As growth continues the hair presently comes to extend beyond the surface of the skin, until much more of it is outside the follicle than in it. At a point on the follicle near the surface certain cells of the Malpighian layer constituting the sheaths bud off groups of cells in which fat droplets accumulate, and which constitute the *sebaceous glands* (Fig.

343). Just proximal to these there also develop, within the dermis, muscle cells which are attached at one end to the outer root sheath and at the other to the under surface of the adjacent epidermis. They are called the *erectile muscles* of the hair, and serve to ruffle it. This helps to keep the animal warm, or probably in other cases to frighten its enemies by making it appear larger, as in the Cat (Fig. 343).

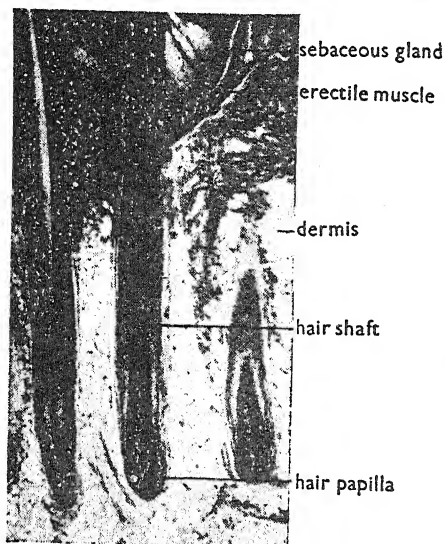


Fig. 343. — Photomicrograph of the same section of hair as in Fig. 342, taken with a lower magnification to show relations to neighboring hairs and also to a sebaceous gland and erectile muscle.

Although not essentially an embryological matter, it is of interest to note that all types of hairs have relatively fixed periods of life. At the end of this period the hair is shed, and its place taken by a new one. As the time for shedding approaches the epidermal cells at the base of the hair shaft and inner root sheath cease dividing. At the same time those constituting the base of the hair become cornified like those in the main part of the shaft. The hair is then detached from the papilla, and easily comes out of the follicle. Later the new hair is formed in the same follicle. The papilla which has shrunk is restored, and the

remaining live epidermal cells which cover it start to multiply. The latter presently give rise to both a new inner sheath and hair shaft in a manner similar to the original process.

NAILS, HOOFS, AND HORNS

It is not feasible to give a discussion of the development of these structures in a volume of this size and character. However it may be noted that once more both nails (claws) and hoofs arise as modifications of epidermal cells, involving mainly their cornification. Horns of one type such as those of the Cow are cornified epidermal sheaths supported by bony cores. The antlers of deer on the other hand are mostly bone covered by a layer of skin (dermis and epidermis) which soon dies

and is rubbed off. The bony horn itself is shed annually, and renewed by a remarkably rapid growth of non-cartilaginous bone. The two last-noted structures are not strictly speaking embryological since they never appear until after birth. Because of their developmental similarity in some respects to the other dermal and epidermal appendages, however, it was thought worth while to mention their origins.

REFERENCES TO LITERATURE

CHAPTERS XIV, XV, XVI AND XVII

- Alden, R. H., "Implantation of the Rat Egg. I. Experimental Alteration of Uterine Polarity," *Jour. Exp. Zool.*, C, 1945.
- Allen, E., "Ovogenesis During Sexual Maturity," *Am. Jour. Anat.*, XXXI, 1923.
- , "The Menstrual Cycle of the Monkey, *Macacus rhesus*: Observations on Normal Animals, the Effects of Removal of the Ovaries and the Effects of Injections of Ovarian and Placental Extracts into the Spayed Animals," *Carnegie Inst. Cont. to Embryology*, XIX, 1927. "Reactions of Immature Monkeys (*Macacus rhesus*) to Injections of Ovarian Hormone," *Jour. Morph. and Physiol.*, XLVI, 1928. — "Further Experiments with an Ovarian Hormone in the Ovariectomized Adult Monkey *Macacus rhesus*, Especially the Degenerating Phase of the Experimental Menstrual Cycle," *Am. Jour. Anat.*, XLII, 1928.
- , Danworth, C. H. and Doisy, E. A., *Sex and Internal Secretions* (2 ed.), Baltimore, 1939.
- Allen, W. M. and Gorner, G. W., "Physiology of the Corpus Luteum. III. Normal Growth and Implantation of Embryos after Very Early Ablation of the Ovaries, under the Influence of Extracts of the Corpus Luteum," *Am. Jour. Physiol.*, LXXXVIII, 1929.
- Anderson, E. L., "The Development of the Pharyngeal Derivatives in the Calf (*Bos taurus*)," *Anat. Rec.*, XXIV, 1922.
- Asdell, S. A., *Patterns of Mammalian Reproduction*, Ithaca, N. Y., 1946.
- Assheton, R., "A Re-investigation into the Early Stages of the Development of the Rabbit," *Q. J. M. S.*, XXXVII, 1894. — "On the Causes which lead to the Attachment of the Mammalian Embryo to the Walls of the Uterus," *Q. J. M. S.*, XXXVII, 1894. — "The Primitive Streak of the Rabbit; the Causes which may determine its Shape and the Part of the Embryo formed by its Activity," *Q. J. M. S.*, XXXVII, 1894. — "The Morphology of the Ungulate Placenta," *Phil. Trans. Roy. Soc.*, CLXXXVIII, 1906. — "The Segmentation of the Ovum of the Sheep, with Observations on the Hypothesis of a Hypoblastic Origin for the Trophoblast," *Q. J. M. S.*, XLI, 1898. — "The Development of the Pig during the First Ten Days," *Q. J. M. S.*, XLI, 1898. — "Early Ontogenetic Phenomena in Mammals," *Q. J. M. S.*, LIV, 1909.
- Baker, B. L., Hook, S. J. and Severinghaus, A. E., "The Cytological Structure of the Human Chorionic Villus and Decidual Parietalis," *Am. Jour. Anat.*, LXXIV, 1944.
- Bartelmez, G. W., "Menstruation," *Physiol. Rev.*, XVII, 1937.
- Bild, A., "Die Entwicklungsgeschichte des Zahnsystem bei *Sus domesticus* und das Verhältniss der Lippenfurchenanlage zur Zahnleiste," *Anat. Anz.*, XX, 1902.
- Blandau, R. J. and Jordan, E. S., "The Effect of Delayed Fertilization on the Development of the Rat Ovum," *Am. Jour. Anat.*, LXVIII, 1941.

- Blandau, R. J. and Money, W. L., "Observations on the Rate of Transport of Spermatozoa in the Female Genital Tract of the Rat," *Anat. Rec.*, XC, 1944.
- , and Young, W. C., "The Effects of Delayed Fertilization on the Development of the Guinea Pig Ovum," *Am. Jour. Anat.*, LXIV, 1939.
- Bonnett, R., "Beiträge zur Embryologie des Hundes: I.," *Anat. Hefte*, IX, 1897. II., *Anat. Hefte*, XVI, 1901; III., *Anat. Hefte*, XX, 1902.
- Bremer, J. L., "I. The Origin of the Pulmonary Arteries in Mammals," *Am. Jour. Anat.*, I, 1902. — "II. On the Origin of the Pulmonary Arteries in Mammals," *Anat. Rec.*, III, 1909. — "The Interrelations of the Mesonephros, Kidney and Placenta in Different Classes of Mammals," *Am. Jour. Anat.*, XIX, 1916. — "Experiments on the Aortic Arches in the Chick," *Anat. Rec.*, XXXVII, 1928. — "The Pneumatization of the Humerus in the Common Fowl and the Associated Activity of Theelin," *Anat. Rec.*, LXXVII, 1940. — "The Pneumatization of the Head of the Common Fowl," *Jour. Morph.*, LXVII, 1940.
- Brewer, J. I., "A Normal Human Ovum in a Stage Preceding the Primitive Streak," *Am. Jour. Anat.*, LXI, 1937.
- Bryce, T. H. and Teacher, J. H., *Contributions to the Study of the Early Development and Imbedding of the Human Ovum. I. An Early Ovum Imbedded in the Decidua*, Glasgow, 1908.
- Burckhard, G., "Die Implantation des Eies der Maus in die Uterusschleimhaut und die Umbildung derselben zur Decidua," *Arch. mikr. Anat.*, LVII, 1901.
- Clements, L. P., "Embryonic Development of the Respiratory Portion of the Pig's Lung," *Anat. Rec.*, LXX, 1938.
- Corner, G. W., "The Structural Unit and Growth of the Pancreas of the Pig," *Am. Jour. Anat.*, XVI, 1914. — "Maturation of the Ovum in Swine," *Anat. Rec.*, XIII, 1917. — "On the Origin of the Corpus Luteum of the Sow from both granulosa and theca interna," *Am. Jour. Anat.*, XXVI, 1919. — "Cyclic Changes in the Ovaries and Uterus of Swine, and their Relations to the Mechanism of Implantation," *Carnegie Inst. Cont. to Emb.*, XIII, 1921. — "The Problem of Embryonic Pathology of Mammals with Observations upon Intra-uterine Mortality in the Pig," *Am. Jour. Anat.*, XXXI, 1923. — "Ovulation and Menstruation in Macacus Rhesus," *Carnegie Inst. Cont. to Emb.*, XV, 1923. — "The Ovarian Hormones and Experimental Menstruation," *Am. Jour. Obs. & Gyn.*, XXXVIII, 1939. — "The Fate of the Corpora Lutea and the Nature of the Corpora Aberrantia in the Rhesus Monkey," *Carnegie Inst. Cont. to Emb.*, XXX, 1942. — *The Hormones in Human Reproduction*, Princeton, 1943.
- , and Allen, W. M., "Physiology of the Corpus Luteum. Part I," *Am. Jour. Physiol.*, LXXXVI, 1928. — "Part II," *Am. Jour. Physiol.*, LXXXVIII, 1929.
- , and Amsbaugh, A. E., "Oestrus and Ovulation in Swine," *Anat. Rec.*, XII, 1917.
- , and Collaborators, "The Physiology of the Corpus Luteum," *Am. Jour. Physiol.*, LXXXVI, LXXXVIII, 1928.
- Coventry, A. F., "The Placenta of the Guinea Baboon (*Cynocephalus papio*, Desmar)," *Anat. Rec.*, XXV, 1923.
- Davis, D. M., "Studies on the Chief Veins in Early Pig Embryos and the Origin of the Vena Cava Inferior," *Am. Jour. Anat.*, X, 1910.
- Doan, C. A., Cunningham, R. S. and Sabin, F. R., "Experimental Studies on the Origin and Maturation of Avian and Mammalian Red Blood Cells," *Carnegie Inst. Cont. to Emb.*, XVI, 1925.
- Dodds, G. S., *The Essentials of Human Embryology*, New York, 1929.
- Fischelis, P., "Beiträge zur Kenntnis der Entwicklungsgeschichte der Gl. thyreoidea und Gl. thymus," *Arch. mikr. Anat.*, XXV, 1885.
- Flint, J. M., "The Development of the Lungs," *Am. Jour. Anat.*, VI, 1906.
- Gilbert, M. S., *Biography of the Unborn*, Baltimore, 1938.

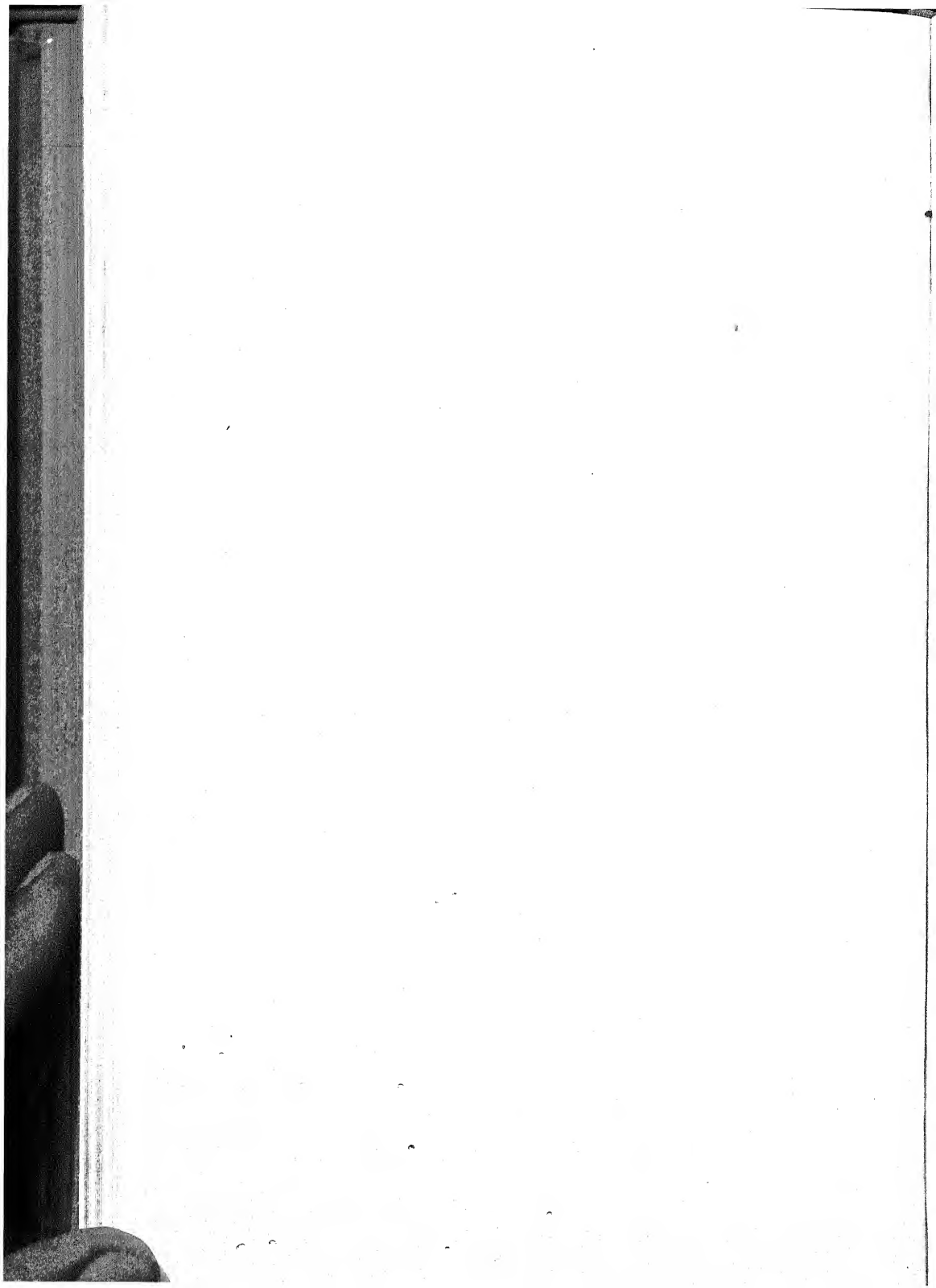
- Godwin, M. C., "The Development of Complex IV in the Pig: a Comparison of the Conditions in the Pig with Those in the Rat, Cat, Dog, Calf, and Man," *Am. Jour. Anat.*, LXVI, 1940.
- Gregory, P. W., "The Early Embryology of the Rabbit," *Carnegie Inst. Cont. to Emb.*, XXI, 1930.
- Gruenewald, P., "The Development of the Sex Cords in the Gonads of Man and Mammals," *Am. Jour. Anat.*, LXX, 1942.
- Hammond, J. and Asdell, S. A., "The Vitality of the Spermatozoa in the Male and Female Reproductive Tracts," *British Journal of Exp. Biol.*, IV, 1926.
- Hargitt, G. T., "The Formation of the Sex Glands and Germ Cells of Mammals." I. "The Origin of the Germ Cells in the Albino Rat," *Jour. Morph. and Physiol.*, XL, 1925. — II. "The History of the Male Germ Cells in the Albino Rat," *Jour. Morph. and Physiol.*, XLII, 1926. — III. "The History of the Female Germ Cells in the Albino Rat, to the Time of Sexual Maturity," IV. "Continuous Origin and Degeneration of Germ Cells in the Female Albino Rat," *Jour. Morph. and Physiol.*, XLIX, 1930.
- Hartman, C. G., "The Homology of Menstruation," *Jour. Am. Med. Assn.*, XCII, 1929. — "How Large is the Mammalian Egg? A Review," *Quart. Rev. Biol.*, IV, 1929. — "How Large is the Human Egg?" *Sci. Am.*, pgs. 214-215, 1930. — "First Findings of Tubal Ova in the Cow, Together with Notes on Oestrus," *Anat. Rec.*, XLVIII, 1931. — "The Follicle-stimulating Effect of Pig Anterior Lobe on the Monkey Ovary," *Anat. Rec.*, L, 1931. — *Time of Ovulation in Women*, Baltimore, 1936.
- , and Corner, G. W., "Removal of the Corpus Luteum and of the Ovaries of the Rhesus Monkey during Pregnancy: Observations and Cautions," *Anat. Rec.*, XCVIII, 1947.
- Heape, W., "The Development of the Mole (*Talpa Europea*). The Formation of the Germinal Layers, and the Early Development of the Medullary Groove and Notochord," *Q. J. M. S.*, XXIII, 1883; XXVII, 1887.
- Henneberg, B., "Anatomie und Entwicklung der Äusseren Genital organe des Schweines und vergleichend-anatomische Bemerkungen. I. Weibliches Schweines," *Zeit. Anat. Entw.*, LXIII, 1922. — "Anatomie und Entwicklung der Äusseren Genital organe des Schweines und vergleichend-anatomische Bemerkungen. Zweiter Teil: Männliches Schweines," *Zeit. Anat. Entw.*, LXXV, 1925. — "Beitrag zur ontogenetischen Entwicklung des Scrotums und der Labia maiora," *Zeit. Anat. Entw.*, LXXXI, 1926.
- Hertig, A. T. and Rock, J., "Two Human Ova of the Pre-villous Stage Having an Ovulation Age of about Eleven and Twelve Days Respectively," *Carnegie Inst. Cont. to Emb.*, XXI, 1941.
- Hertwig, O., *Lehrbuch der Entwicklungsgeschichte des Menschen und der Wirbeltiere* (9 ed.), Jena, 1910. — (Editor) *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere*, Jena, 1906.
- J. P., "Contributions to the Embryology of the Marsupialia. I. The Placentation of *Perameles*," *Q. J. M. S.*, XL, 1897. — "On the Fœtal Membranes Placentation, and Parturition of the Native Cat (*Dasyurus viverrinus*)," *Anat. Anz.*, XVIII, 1900.
- Heuser, C. H., "The Development of the Cerebral Ventricles in the Pig," *Am. Jour. Anat.*, XV, 1913. — "The Branchial Vessels and Their Derivatives in the Pig," *Carnegie Inst. Cont. to Emb.*, XV, 1923. — "A Study of the Implantation of the Ovum of the Pig from the Stage of the Bilaminar Blastocyst to the Completion of the Fetal Membranes," *Carnegie Inst. Cont. to Emb.*, XIX, 1926.
- , and Streeter, G. L., "Early Stages in the Development of Pig Embryos, from the Period of Initial Cleavage to the Time of the Appearance of Limb-buds," *Carnegie Inst. Cont. to Emb.*, XX, 1929.

- Hill, E. C., "On the First Appearance of the Renal Artery and the Relative Development of Kidneys and Wolffian Bodies in Pig Embryos," *Johns Hopkins Bull.*, XVI, 1905.
- Hirsch, M., "Der Lückzahn von *Sus domesticus*, ein Beitrag zur Entwicklungsgeschichte des Gebisses von *Sus domesticus* und zur Kenntnis des Wesens der Dentitionen," *Anat. Anz.*, LIV, 1921.
- Hisaw, F. L., "The Corpus Luteum Hormone." I. "Experimental Relaxation of the Pelvic Ligaments of the Guinea-Pig," *Physiol. Zool.*, II, 1929.
- , Fevold, H. L. and Meyer, R. K., "The Corpus Luteum Hormone." II. "Methods of Extraction," *Physiol. Zool.*, III, 1930. — "Production of a Premenstrual Endometrium in Castrated Monkeys by Ovarian Hormones," *Proc. Soc. Exp. Biol. and Med.*, XXVII, 1930.
- , and Leonard, S. L., "Relation of the Follicular and Corpus Luteum Hormones in the Production of Progestational Proliferation of the Rabbit Uterus," *Am. Jour. Physiol.*, XCII, 1930.
- , Creep, R. O. and Fevold, H. L., "The Effects of Oestrin-progestin Combinations on the Endometrium, Vagina and Sexual Skin of Monkeys," *Am. Jour. Anat.*, LXI, 1937.
- Hubrecht, A. A. W., "The Placentation of the Shrew (*Sorex vulgaris*)," *Q. J. M. S.*, XXXV, 1893-1894. — "Die Phylogenese des Amnions und die Bedeutung des Trophoblastes," *Verhand. Kon. Acad. Wetensch.*, Amsterdam, VIII, 1902. — "Die Keimblase von *Tarsius*," *Festsch. f. Gegenbaur.*, Leipzig, 1896. — "Ueber die Entwicklung der Placenta von *Tarsius* und *Tupaia*," *Proc. Internat. Cong. Zool.*, Cambridge, 1899. — "Early Ontogenetic Phenomena in Mammals and Their Bearing on our Interpretation of the Phylogeny of the Vertebrates," *Q. J. M. S.*, LIII, 1908.
- Jenkinson, J. W., "Observations on the Histology and Physiology of the Placenta of the Mouse," *Tijds. Nederl. Dierk. Ver.*, II, vii, 1902. — "Notes on the Histology and Physiology of the Placenta in Ungulata," *Proc. Zool. Soc.*, 1906. — *Vertebrate Embryology*, Oxford and London, 1913.
- Johnstone, R. W., "The New Physiology of Menstruation, and its Practical Implications in Obstetrics and Gynecology," *Am. J. Obs. and Gynec.*, XIX, 1930.
- Keibel, F., "Studien zur Entwicklungsgeschichte des Schweines (*Sus scrofa domestica*)," II. *Morph. Arbeiten*, V, 1896. — "Normentafeln zur Entwicklungsgeschichte der Wirbelthiere," I, "Normentafel zur Entwicklungsgeschichte des Schweines (*Sus scrofa domestica*)," Jena, 1897. "Zur vergleichenden Keimesgeschichte der Primaten," *Selenka's Studien über Entwicklungsgeschichte der Tiere*, X, Wiesbaden, 1903. — (With Mall, E. P. — Editors and contributors) *Handbuch der Entwicklungsgeschichte des Menschen*, Leipzig, 1910, 1911. American Edition: *Manual of Human Embryology*, Philadelphia, 1910, 1912.
- Kirkham, W. B., "Maturation of the Egg of the White Mouse," *Transactions of the Connecticut Academy of Arts and Sciences*, XIII, 1907 (See also *Biol. Bull.*, XII, 1907). — "Ovulation in Mammals, with Special Reference to the Mouse and Rat," *Biol. Bull.*, XVIII, 1910.
- Klaatsch, H., "Ueber den Descensus testicularum," *Morph. Jahrb.*, XVI, 1890.
- Klapper, C. E., "The Development of the Pharynx of the Guinea Pig with Special Emphasis on the Morphogenesis of the Thymus," *Am. Jour. Anat.*, LXXVIII, 1946. — "The Development of the Pharynx of the Guinea Pig with Special Emphasis on the Fate of the Ultimobranchial Body," *Am. Jour. Anat.*, LXXIX, 1946.
- Kolliker, A. von, *Entwicklungsgeschichte des Menschen und der höheren Thiere* (2 ed.), Leipzig, 1876, 1879. — *Grundriss der Entwicklungsgeschichte des Menschen und der höheren Thiere* (2 ed.), Leipzig, 1884.

- Kollmann, J., "Die Körperform menschlicher normaler und pathologischer Embryonen," *Arch. Anat. Physiol.*, Supplement, 1899. — *Handatlas der Entwicklungsgeschichte des Menschen*, Jena, 1907.
- Lewis, F. T., "The Gross Anatomy of a 12-mm. Pig," *Am. Jour. Anat.*, II, 1902. — "The Fifth and Sixth Aortic Arches and the Related Pharyngeal Pouches in the Rabbit and Pig," *Anat. Anz.*, XXVIII, 1906.
- Lineback, P. E., "The Development of the Spiral Coil in the Large Intestine of the Pig," *Am. Jour. Anat.*, XX, 1916.
- Lockwood, C. B., Development and Transition of the Testis, Normal and Abnormal," *Jour. Anat. Physiol.*, Part I, XXI, Part II, XXII, Part III, XXII, Part IV, XXII, 1888.
- Loeb, L., "Beiträge zur Analyse des Gewebewachstums: III. Die Erzeugung von Deciduen in dem Uterus des Kaninchens," *Arch. Entw.-mech.*, XXVII, 1909. — "The Function of the Corpus Luteum, the Experimental Production of the Maternal Placenta, and the Mechanism of the Sexual Cycle in the Female Organism," *Medical Record*, LXXVII, 1910.
- Longley, W. H., "The Maturation of the Egg and Ovulation in the Domestic Cat," *Am. Jour. Anat.*, XII, 1911.
- Mall, F. P., "The Development of the Lesser Peritoneal Cavity in Birds and Mammals," *Jour. Morph.*, V, 1891.
- Markee, J. E. and Hinsey, J. C., "A Case of Probable Superfetation in the Cat," *Anat. Rec.*, LXI, 1935. — "Studies on Uterine Growth. II. A Local Factor in the Pregnant Uterus in the Cat," *Anat. Rec.*, LXI, 1935.
- Marshall, F. H. A., *The Physiology of Reproduction*, London (2 ed.), 1922. — *An Introduction to Sexual Physiology*, New York, 1925.
- Martin, C. P. and Falkiner, N. McL., "The Falkiner Ovum," *Am. Jour. Anat.*, LXIII, 1938.
- McClure, C. F. W., "The Development of the Lymphatic System in the Light of the More Recent Investigations in the Field of Vasculogenesis," *Anat. Rec.*, IX, 1915.
- Mead, C. S., "The Chondrocranium of an Embryo Pig, *Sus scrofa*. A Contribution to the Morphology of the Mammalian Skull," *Am. Jour. Anat.*, IX, 1909.
- Minot, C. S., *Human Embryology*, New York, 1892. — "A Bibliography of Vertebrate Embryology," *Mem. Boston Soc. Nat. Hist.*, IV, 1893. — *A Laboratory Text Book of Embryology* (2 ed.), Philadelphia, 1911.
- Moody, R. O., "Some Features of the Histogenesis of the Thyroid Gland in the Pig," *Anat. Rec.*, IV, 1910.
- Morrill, C. V., "On the Development of the Atrial Septum and the Valvular Apparatus in the Right Atrium of the Pig Embryo, with a Note on the Fenestration of the Anterior Cardinal Veins," *Am. Jour. Anat.*, XX, 1916.
- Nelson, W. O. and Haterius, H. O., "An Experimental Study of Ovariectomy and Transplantation in the Albino Rat," *Physiol. Zool.*, III, 1930.
- Noback, C. R., "Placentation and Angiogenesis in the Amnion of a Baboon (*Papio papio*)," *Anat. Rec.*, XCIV, 1946.
- Papanicolaou, G. N. and Blau, N. F., "The Existence of a Sexual Rhythm and Experimental Induction of Heat in the Dog during Anæstrus," *Anat. Rec.*, XXXV, March, 1927.
- Parker, G. H., "Passage of Sperms and Eggs Through Oviducts in Terrestrial Vertebrates," *Phil. Trans. Roy. Soc.*, Series B, CCXIX, 1931.
- Parker, W. K., "On the Structure and Development of the Skull of the Pig," *Phil. Trans. Roy. Soc.*, Series B, CLXIV, 1874.
- Patten, B. M., *The Embryology of the Pig* (2 ed.), Philadelphia, 1931. — *Human Embryology*, Philadelphia, 1946.

- Peters, H., *Ueber die Einbettung des menschlichen Eies und das früheste bisher bekannte menschliche Placentationsstadium*, Leipzig and Wien, 1899.
- Phillips, R. W. and Andrews, F. N., "The Speed of Travel of Ram Spermatozoa," *Anat. Rec.*, LXVIII, 1937.
- Rabl, C., *Die Entwicklung des Gesichtes: Das Gesicht der Säugethiere*, Leipzig, 1902.
- Rawn, E., "Ueber die Entwicklung des Septum transversum," *Anat. Anz.*, XV, 1899.
- Robinson, A., "Observations upon the Development of the Segmentation Cavity, the Archenteron, the Germinal Layers, and the Amnion in Mammals," *Q. J. M. S.*, XXXIII, 1892. — "The Early Stages of the Development of the Pericardium," *Jour. Anat. Physiol.*, XXXVII, 1903.
- Rossman, I., "Uterine Contractions and the Transport of Sperm in the Rat," *Anat. Rec.*, LXIX, 1937.
- Sabin, F. R., "On the Origin of the Lymphatic System from the Veins and the Development of the Lymph Hearts and Thoracic Duct in the Pig," *Am. Jour. Anat.*, I, 1902. — "The Origin and Development of the Lymphatic System," *Johns Hopkins Hosp. Rep.*, XVII, 1916. — "Origin and Development of the Primitive Vessels of the Chick and of the Pig," *Carnegie Inst. Cont. to Emb.*, VI, 1917.
- Schmidt, V., "Studien über die Histogenese der Haut und ihrer Anhangsgebilde bei Säugetieren und beim Menschen. I. Die Histogenese des Hufes bei Schweine-embryonen," *Zeit. mikr.-Anat. Forsch.*, III, 1925.
- Schoenfeld, H., "Contribution a l'Etude de la Fixation de l'œuf des Mammifères dans la cavité utérine, et des premiers stades de la Placentation," *Arch. Biol.*, XIX, 1903.
- Schott, R. G., "Rate of Sperm Travel and Time of Ovulation in Sheep," *Anat. Rec.*, LXXIX, 1941.
- Selenka, E., *Studien über Entwicklungsgeschichte der Thiere: IV. Das Opossum, 1887; V 1. Beutelfuchs und Känguruhratte; zur Entstehungsgeschichte der Amnion der Kantjil (Tragulus javanicus); Affen Ost-Indiens, 1891; V 2. Keimbildung des Kalong; Dottersack und placenta des Kalong, 1892.*
- Semon, R., "Die Embryonalhüllen der Monotermen und Marsupialier," *Zoöl. Forschungsreise in Australien*, II.
- Sobotta, J., "Die Befruchtung und Furchung des Eies der Maus," *Arch. mikr. Anat.*, XLV, 1895 (For corrections, see Kirkham). — "Die Furchung des Wirbeltiereies," *Ergeb. Anat. u. Entw.*, VI, 1896 (1897). — "Die Bildung der Richtungskörper bei der Maus," *Anat. Hefte*, XXXV, 1907.
- Soderwall, A. L. and Blandau, R. J., "The Duration of the Fertilizing Capacity of Spermatozoa in the Female Genital Tract of the Rat," *Jour. Exp. Zoöl.*, XXCVIII, 1941.
- , and Young, W. C., "The Effect of Aging in the Female Genital Tract on the Fertilizing Capacity of Guinea Pig Spermatozoa," *Anat. Rec.*, LXXVIII, 1940.
- Spee, F., Graf von, "Beobachtung an einer menschlichen Keimscheibe mit offener Medullarrinne und Canalis neurentericus," *Arch. Anat. u. Physiol.*, 1889. — "Neue Beobachtungen über sehr frühe Entwicklungsstufen des menschlichen Eies," *Arch. Anat. u. Physiol.*, 1896. — "Die Implantation des Meerschweincheiees in die Uteruswand," *Zeitschrift für Morphologie und Anthropologie*, III, 1901.
- Strahl, H. and Happe, H., "Ueber die Placenta der Schwanzaffen," *Selenka's Studien über die Entwicklungsgeschichte der Tiere*, XIII, Wiesbaden, 1905.
- Streeter, G. L., "On the Development of the Membranous Labyrinth and the Acoustic and Facial Nerves in the Human Embryo," *Am. Jour. Anat.*, VI, 1907.

- "The Miller Ovum—the Youngest Normal Human Embryo thus far Known," *Carnegie Inst. Cont. to Emb.*, XVIII, 1926. — "Development of the Mesoblast and Notochord in Pig Embryos," *Carnegie Inst. Cont. to Emb.*, XIX, 1927. — "Characteristics of the Primate Egg Immediately Preceding Its Attachment to the Uterine Wall," *Carnegie Inst. of Wash.*, Pub. No. 501, 1938.
- Thoms, H., "Untersuchungen über Bau, Wachstum und Entwicklung des Hufes der Artiodactylen, insbesondere des *Sus scrofa*," *Deutsche Thierärztliche Wochenschr.*, IV, 1896.
- Thyng, F. W., "Models of the Pancreas in Embryos of the Pig, Rabbit, Cat and Man," *Am. Jour. Anat.*, VII, 1908. — "The Anatomy of a 7.8-mm. Pig Embryo," *Anat. Rec.*, V, 1911.
- Turner, W., *Lectures on the Comparative Anatomy of the Placenta*, Edinburgh, 1876.
- Tuttle, A. H., "The Relation of the External Meatus, tympanum and Eustachian Tube to the First Visceral Cleft," *Proc. Am. Acad. Arts and Sci.*, XIX, 1884.
- Van Beneden, E., "Recherches sur l'embryologie des mammifères: La formation des feuillets chez le Lapin," *Arch. Biol.*, I, 1880. — "Recherches sur les premiers stades du développement du Murin (*Vesperilio murinus*)," *Anat. Anz.*, XVI, 1899. — (Brachet, editor) "Recherches sur l'embryologie des Mammifères: I. De la segmentation, de la formation de la cavité blastodermique et de l'embryon didermique chez le Murin," *Arch. Biol.*, XXVI, 1911. — "II. De la ligne primitive, du prolongement cephalique de la notochorde et du mésoblaste chez la lapin et chez le murin," *Arch. Biol.*, XXVII, 1912.
- Webster, C., *Human Placentation*, Chicago, 1901.
- Weller, G. L., Jr., "Development of the Thyroid, Parathyroid and Thymus Glands in Man," *Carnegie Inst. Cont. to Emb.*, XXIV, 1933.
- Whitehead, R. H., "The Histogenesis of the Adrenal in the Pig," *Am. Jour. Anat.*, II, 1903.
- Wieman, H. L., *An Introduction to Vertebrate Embryology*, New York, 1930.
- Wimsatt, W. A., "Further Studies on the Survival of Spermatozoa in the Female Reproductive Tract of the Bat," *Anat. Rec.*, XXCVIII, 1944.
- Winiwarter, H. von, "Recherches sur l'ovogenèse et l'organogenèse de l'ovaire des Mammifères (Lapin et Homme)," *Arch. Biol.*, XVII, 1901. — "Nachtrag zu meiner Arbeit über Oögenese der Säugetiere," *Anat. Anz.*, XXII, 1902.
- Wislocki, G. B., "Hemopoiesis in the Chorionic Villi of the Placenta of Platyrrhine Monkeys," *Anat. Rec.*, LXXXV, 1943. — "Histochemical Reactions of the Placenta of the Pig," *Am. Jour. Anat.*, LXXVIII, 1946.
- , and Bennett, H. S., "The Histology and Cytology of the Human and Monkey Placenta, with Special Reference to the Trophoblast," *Am. Jour. Anat.*, LXXXIII, 1943.
- Wright, P. L., "Delayed Implantation in the Long-tailed Weasel (*Mustela frenata*) the Short-tailed Weasel (*Mustela Cicognani*), and the Marten (*Martes americana*)," *Anat. Rec.*, LXXXIII, 1942.
- Zeitzschmann, O., "Die Entwicklung des Systems der äusseren Haut. (b) Die Haare. (Schwein)," *Lehrbuch der Entwicklungsgeschichte der Haustiere* S. 186-194, 1924.



INDEX

Page numbers in *italics* indicate a definition or special reference; those in heavy-face type indicate illustrations.

- Abel, S., 459
 Abraxas type,
 sex chromosomes in, 35
 acrosome, 11
 Adelmann, H. B., 161
 adrenals, in Frog, 232, **232**, 233; in
 Chick, 428, 475; in Pig, 644
 cortex of, 645
 medulla of, 645
 air capillaries, 443
 air chamber, in Chick egg, 286
 air sacs, in Chick, 440, 443, 444
 abdominal, 444, 444, 445
 cervical, 444, 444
 interclavicular, 444, 444
 intermediate, 444
 alae, in Pig, 609
 Albaum, H. G., 194
 albuginea, 5, in Chick, 471, 472
 albumen, in Chick, 282, 286, 366
 dense, source of, 288, 289
 thin, source of, 288, 289
 albumen-sac, in Chick, 364, 365, 366
 Alden, R. H., 541
 Alexander, L. E., 354
 alimentary tract, in Frog, 162, 163,
 200-208; in Chick, 335-338, 371-
 377, 398-401, 442-449
 sources of, 67
 allantoic cavity, in Pig, 535
 allantoic placenta, in Marsupials, 532,
 533, 533
 allantoic stalk, in Chick, 364, 364, 365,
 449; in Pig, 568, 569, 582, 583,
 583, 645, 646, 647
 allantois, in Chick, 360, 361, 362, 363,
 363, 364, 365, 366, 375, 376, 376,
 377, 445, 448, 455, 457, 476; in
 Mammal, 529; Monotremes, 530,
 531, 532, 533; Cat, 538, 539, 540;
 Rabbit, 543; Primates, 546, 547,
 548, 550; Pig, 534, 535, 536, 536,
 537, 548, 563, 564, 574, 575, 576
 source of blood corpuscles in, in Pig,
 586
 Allen, B. M., 6, 174
 Allen, E., 491
 alveoli, 632
 Amblystoma, 160, 168, 188, 239
 ameloblasts, in Pig, 658, 659, 660, 661,
 662
 ameloblast layer, in Pig, 623
 amnio-cardiac vesicles, in Chick, 306,
 321, 326, 339, 341; in Pig, 558
 amnion, in Chick, 359, 359, 360, 361,
 364, 365, 380, 476
 formation of, 358-361
 amnion, in Mammal, Primates, 546,
 547, 550; (Man and Apes), 557;
 Pig, 535, 562, 563, 564, 569, 571
 formation of, in Chick, 358-361; in
 Pig, 515, 515, 517
 methods I and II compared, 522-525
 methods of formation, in Reptiles
 (Sauropsids), 523; in Mammals
 (I), 514, 515, 515, 516, 517, (II),
 517, 518, 519, 520, 521
 amniota, 357
 amniotic cavity, in Chick, 359, 360, 364,
 365; in Mammal, Monotremes,
 530; Marsupials, 530; Primates,
 546, 547, 548
 amniotic fluid, in Chick, 360
 amniotic folds, in Chick, 334, 358, 358;
 in Pig, 574
 amniotic umbilicus, in Chick, 359
 Amphibians, 38, 60, 132, 133, 189, 190,
 219, 238, 239
 Amphioxus, 75
 Amphioxus and Frog,
 summary of early development, 144-
 146
 Amprino, R., 416
 ampulla, in Frog ear, 193, 194; in
 Chick, 389, 422, 422, 423
 of gonads, in Frog, 237, 238
 anal plate, in Chick, 338, 338, 376,
 377, 377, 448; in Pig, 574, 581,
 664
 anamniota, 357
 Anasa tristis, spermatogenesis in, 31
 Andrews, F. N., 506

- androgamones, 39
- animal pole, of egg, 8, 10, 55; in Amphioxus, 79, 80, 82, 84, 85; in Frog, 109, 110, 117, 123, 125, 129; in Fish, 262
- annulus tympanicus, in Frog, 196, 249
- anoestrus, in Mammal, 495, 496, 501
- anterior chamber of eye, in Chick, 418, 421
- anus, in Frog, 207, 208; in Gymnophiona, 275; in Chick, 315, 449; in Pig, 583, 645, 648, 653
- aorta or artery, dorsal or main systemic, in Frog, 219, 219; in Teleost, 274; in Chick, 341, 343, 344, 373, 378, 404, 451, 452, 452, 453, 453, 454; in Pig, 536, 569, 576, 578, 587, 590, 591, 593, 594, 595, 635
- ventral, in Chick, 342, 343, 344, 344; in Pig, 569
- aortic arch or arches, in Frog, 189, 202, 216, 217; in Chick, 334, 344, 345, 346, 373, 378, 378, 379, 380, 403, 404, 404, 452, 452, 453, 453, 454, 454, 461; in Pig, 576, 590, 591, 592, 593, 593, 635, 636
- alterations in at hatching or birth, 454, 455
- reasons for disappearance of left fourth in Bird, 453, 454, 454
- aqueduct of Sylvius, in Frog, 181; in Chick, 413; in Pig, 612
- aqueous humor, in Chick, 421
- archenteron, 53, 55; in Frog, 130, 131, 133, 157; in Teleost, 265, 265, 271; in Gymnophiona, 275, 277, 277; in Chick, 302, 302; in Mammal, 510, 512, 513; Pig, 508
- area opaca, in Chick, 301, 301, 302, 302
- area pellucida, in Chick, 294, 301, 301, 302, 302, 318, 322
- area vasculosa, in Chick, 317, 322, 345, 346, 347; in Mammals, Marsupials, 531, 532; Pig, 534, 585
- area vitellina externa, in Chick, 317, 318, 322
- area vitellina interna, in Chick, 306, 318, 318, 322
- areolae, in Pig, 536
- artery or arteries, allantoic, in Chick, 364, 406, 407
- basilar, in Pig, 569, 592, 593, 594, 595
- brachiocephalic (innominate), in Pig, 594, 635
- carotid, common, in Chick, 404, 452, 453, 454, 457; in Pig, 592, 594, 635, 637; external, in Frog, 219, 219; in Chick, 346, 380, 403, 404, 452, 452, 453, 454, 457, 460, 461; in Pig, 592, 593, 594, 595, 635; internal, in Frog, 203, 219, 219; in Chick, 346, 380, 403, 404, 452, 452, 453, 454, 457, 460, 461; in Pig, 593, 594, 595, 635
- caudal, in Chick, 378; in Pig, 569, 593
- central, of retina, in Pig, 617
- coeliac, in Chick, 457, 460, 461; in Pig, 593, 596, 637
- hyaloid, in Pig, 617
- iliac, in Frog, 220
- common, in Pig, 637
- external, in Pig, 637
- internal, in Pig, 637
- intestinal, in Pig, 536
- lingual, in Frog, 218, 219
- lumbar, in Frog, 220
- mammary, in Pig, 636, 637
- mesenteric, in Frog, 220; in Chick, 457, 460, 461; in Pig, 593, 596, 637
- palatine, in Frog, 218
- pharyngeal, in Frog, 218, 220
- pulmonary (or pulmo-cutaneous in Frog), in Frog, 218, 219; in Chick, 404, 404, 451, 451, 452, 453, 456, 457, 460, 461; in Pig, 591, 592, 593, 594, 594, 636, 641
- renal, in Chick, 406; in Pig, 596, 637, 644
- sciatic, in Chick, 404, 406, 407, 457, 461, 463
- segmental (or intersegmental), in Chick, 343, 396; in Pig, 590, 591, 593, 595, 636
- spinal, in Pig, 587
- subclavian, in Frog, 220; in Chick, 404, 404, 451, 452, 453, 457, 460, 461; in Pig, 591, 592, 593, 594, 595, 635, 636, 637
- umbilical, in Chick, 406, 457, 461, 463; in Pig, 536, 569, 582, 583, 593, 596, 605
- vertebral, in Pig, 592, 593, 594, 595
- vitelline, in Chick, 333, 343, 347, 379.

- 382, 407, 408, 457, 463; in Pig, 536, 569, 582, 595
- Ascaris, 23
- Aschheim-Zondek test for pregnancy, 503
- Asdell, J. A., 504, 507
- Asmundsen, V. S., 289
- astrocytes, in Pig, 567
- atrio-ventricular aperture, in Frog, 221
- atrio-ventricular canal, in Chick, 401, 450; in Pig, 589, 641
- atrio-ventricular valves, in Chick, 459 (See mitral and tricuspid valves in Pig)
- atrium or atria, in Frog, 178, 212, 213; in Chick, 341, 342, 378, 379, 380, 384, 401, 402, 403, 451; in Pig, 536, 569, 578, 587, 588, 588, 640, 641
- Atwell, W. J., 174
- auditory capsule or vesicle, in Frog, 189, 193, 195, 248, 249, 250; in Chick, 353, 355, 379, 421, 422, 423, 440; in Pig, 571, 573
- auditory organ, in Pig, 617
- auditory pit, in Chick, 355; in Pig, 564
- auditory placode, in Frog, 159, 165
- auricles of heart, in Chick, 451; in Pig, 643
- auricular-rump axis, in Pig, 563
- autonomic ganglia or nerves (See under nerves)
- axes, of Frog egg, 115
- axial filament, of sperm, 12
- Axolotl, 189; gastrulation in, 137, 139; maturation of germ cells in, 18, 19
- Bacon, R. L., 168
- Baker, B. L., 503
- Bakst, H., 460
- balancers, in Amblystoma, 161
- Ballard, W. W., 133
- Bandicoot (See Perameles)
- Bang, A., 343
- barbs, in Chick feathers, 436
- Barclay, A. E., 458
- Barcroft, J., 458
- Barron, D. H., 458
- Bartelmez, G. W., 323
- Barth, L. G., 141, 143
- basiscranial fontanelle, in Frog, 250
- basil or basilar plate, in Frog, 248; in Chick, 440; in Pig, 618, 655
- Bat, 508
- Bautzmann, H., 139
- Becker, R. F., 458
- Beckwith, C. J., 190
- Bidder's organ, in Frog, 238
- bile duct, in Frog, 206; in Chick, 374, 374, 446; in Pig, 569, 580 (See also ductus choledochus)
- Birds, sex determination in, 38
- bladder, urinary, in Pig, 604
- Blandau, R. J., 507, 508
- blastema cells, mesonephric, in Frog, 233
- blastocoel or segmentation cavity, 53, 55, 55; in Amphioxus, 88; in Frog, 124, 124, 125, 130, 131, 132, 133; in Teleost, 263, 264; in Chick, 294, 295, 296, 297; in Mammal, Primates, 548, 548, 549, 549; Pig, 508, 575
- blastocyst, in Mammal, 509, 510, 510, 521; Ungulates, 534; Carnivores, 539; Primates, 548, 548, 549, 549, 553, 556; early Human, 552, 553; Pig, 535, 535 (See also blastodermic vesicle)
- distribution in horns of uterus, Pig, 537
- blastoderm, 53; in Teleost, 263; in Chick, 286, 294, 298, 306; in Mammal, 510; Primates, 548, 549; Pig, 522, 524, 525
- homologies of margin, in Chick, 303, 318, 319
- potentialities, in Chick, 312, 313
- blastodermic vesicle, in Pig, 509, 510, 511, 535, 535
- blastodisc, 10, 53; in Teleosts, 262, 263
- blastoporal lip, 55, 55; in Amphioxus, 88, 89, 90; in Frog, 127, 128, 129, 130, 131, 132, 133, 134; in Teleost, 265, 265, 266, 266, 267, 268, 268; in Gymnophiona, 274, 275
- homologies of, in Chick, 298, 299, 303, 314, 318, 319
- blastopore, 55, 55; in Amphioxus, 88, 89, 90; in Frog, 129, 130, 131, 152; in Teleosts, 266, 267; in Gymnophiona, 273; in Chick, 314, 318; in Mammal, 513, 526, 527
- blastula, 53; in Amphioxus, 87; in Frog, 117, 124, 124, 132; in Teleost, 263; in Chick, 298
- blood corpuscles, source of, in Frog, 216; in Chick, 316, 340; in Pig, 586

- blood islands, in Frog, 216; in Chick, 305, 306, 307, 316, 322; in Pig, 585
- blood system, source of, 67
- blood vessels, of bone in Frog, 246, 252; placental in Man, 554, 555; of villi in Pig, 586
- "blue babies," cause of, 458
- body cavities, in Chick, 465, 466; in Pig, 633, 634
- body shape, in Chick, fifth day, 433
- bone,
 cancellous, in Frog, 241, 244, 245; in Chick, 439, 440; in Pig, 657
 compact, in Frog, 242, 245
 dermal, in Frog, 250, 251
 endochondral or cartilage, in Frog, 244, 245; in Chick, 439, 439, 440, 441; in Pig, 655, 656, 657
 histogenesis, in Frog, 240-246, 252, 253, 254; in Chick, 439, 440; in Pig, 656, 657
 lamella, 245, 246, 252
 membrane or membranous, in Frog, 245, 246; in Chick, 439, 441; in Pig, 655
 periosteal, 245, 245, 246, 252; in Chick, 439, 439; in Pig, 656
 skull (*See* skull)
 trabeculae of mandible, in Pig, 623
- bones, of limbs, in Frog, 252, 253, 254; in Chick, 438, 439, 440; in Pig, 657, 658
- bony labyrinth, in Chick, 423; in Pig, 619
- Bowman's capsule, in Frog, 232; in Chick, 390; in Pig, 604, 644
- Brachet, A., 59, 109, 133
- brain,
 development and divisions of, in, Frog, 156, 157, 158, 176, 177, 177, 178, 179, 180; in Chick, 348-351, 348, 349, 383, 384, 384, 409-413, 410, 411, 412; in Pig, 566, 567, 610-613, 610
 lobes of, in Pig, 610
- branchial or gill,
 arch, in Frog, 150, 153, 163, 164, 201, 201, 217, 218, 219
 chamber, in Frog, 172, 202, 203
 circulation, in Frog, 202, 216, 217
 cleft or clefts, in Frog, 150, 151, 160, 169, 170, 201
 plate, in Frog, 148, 150, 151
- pouches, 160, 162, 201, 201, 202, 204, 205, 205
- rakers, in Frog, 203, 204
- breeding season, in Mammals, 496
- Bremer, J. L., 453
- Brewer, J. I., 552
- Brizee, K. R., 387
- broad ligament (mesovarium), in Pig, 647, 648, 653
- bronchial tubes, in Pig, 632
- bronchus or bronchi, in Chick, 373, 443; in Pig, 577, 579, 632
- primary, secondary, and tertiary, in Chick, 413, 443, 444
- recurrent, in Chick, 444, 445
- Bruner, J. A., 239
- Bueker, E. D., 386
- bulbo-conus (arteriosus), in Pig, 536, 587
- bulbo-urethral gland, in Pig, 645, 646, 648
- bulbus arteriosus, in Frog, 212, 214; in Chick, 341, 342, 342, 346, 378, 379, 380, 384, 401, 402, 450, 451
- Burmester, B. N., 289
- Burns, R. K., 239
- bursa Fabricii, in Chick, 448, 449, 449
- Bryce-Teacher, blastocyst, 552
- caecum, intestinal, in Pig, 581, 627, 629
- Cairns, J. M., 436
- Calkins, G. N., 48
- Cameron, J. A., 216
- canals, of Gärtner, in Pig, 649, 653
- Carnivores,
 allantois in, 538, 540
 implantation in, 540
 placenta in, 540
 pro-oestrus in, 537
 yolk-sac in, 537, 538, 538
- carotid gland, in Frog, 204, 205, 205
- carotid loop, in Chick, 334, 343
- cartilage,
 arytenoid, in Pig, 624
 basal, or basilarplate, in Frog, 248; in Chick, 440; in Pig, 655
 basibranchial, in Frog, 250
 basihyal, in Frog, 250
 basioccipital, in Chick, 441
 ceratohyal, in Frog, 251, 253
 cricoid, in Pig, 624
 diaphysial, in Chick, 439
 epiphysial, in Frog, 243; in Chick, 43
 exoccipitals, in Chick, 441

- hypobranchials, in Frog, 250, 251
 in bone-making, 243, 243, 244
 mandibular, in Pig, 621
 matrix, in Frog, 244
 Meckel's, in Frog, 248, 251; in Chick, 441; in Pig, 655
 mesotic, in Frog, 248, 250
 nasal, in Pig, 621
 occipital, in Frog, 250
 olfactory, 249
 palato-quadrate, in Frog, 203, 248, 249, 251; in Chick, 441
 parachordals (or parachordal plate), in Frog, 248, 248; in Chick, 440; in Pig, 655
 thyroid, in Pig, 624
 trabecular, in Frog, 203, 248, 250; in Chick, 440; in Pig, 655
 vertebral, in Frog, 247, 247
 caruncles, in Mammals, Ungulates, 536
 Cat,
 allantois in, 538, 540
 amnion in, 538
 egg of, 505
 ovulation in, 493
 parturition stimulus in, 504
 placenta in, 538, 540, 593
 sexual cycle in, 496
 yolk-sac in, 538
 caudal flexure, in Chick, 370, 395; in Pig, 562, 566
 caudal knob, in Teleost, 269
 caval fold, in Chick, 406
 cell layer, of Langhans, in Mammals, Man and Apes, 554, 555, 556
 cells,
 central, in Chick blastoderm, 294, 295, 296
 chromaffin, in Chick, 475; in Pig, 645
 marginal, in Chick blastoderm, 294, 295, 296
 of Rauber, in Mammals, Rabbit, 514; Pig, 516
 cement, of tooth, 551
 cementum, of tooth, 660
 central body, 10
 centriole, 11, 12
 centrosome, 11, 12, 17; in Frog egg, 108; in Chick egg, 285
 centrum, in Frog, 247, 247; in Chick, 437; in Pig, 655
 cerebellum, in Frog, 181; in Chick, 412, 413; in Pig, 610, 613
 cerebral suture, anterior, in Chick, 328
 Cerebratulus, loss of chromatin in, 27
 cerebrum or cerebral hemispheres, in Frog, 177; in Chick, 350, 409, 410, 411, 412, 413; in Pig, 610, 610, 611
 Cerfontaine, P., 75
 cervical flexure, in Chick, 333, 333, 334, 370, 379, 395, 409, 433; in Pig, 562, 566
 cervical sinus, in Pig, 563, 565
 cervix, in Pig, 649
 chalazae, in Chick, 286
 source of, in Chick, 288, 289, 290
 chalaziferous membrane, in Chick, 286
 source of, in Chick, 286, 288, 290
 Chang, C. Y., 239
 Chen, B. K., 300
 chiasma (chiasmotypy), 20, 23, 24
 chondrin, 243, 244
 chondrioblasts, 244
 chordae tendineae, in Pig, 641, 642
 chorio-allantoic membrane, in Chick, 364
 chorion (false amnion), in Chick, 360, 361, 364, 364, 365, 366, 380; in Mammal, 513, 517; Monotremes, 530; Cat, 539; Man and Apes, 546, 547, 554, 555, 556, 556; Pig, 537, 563, 564
 frondosum, in Mammal, Man and Apes, 557, 559
 laeve, in Mammal, Man and Apes, 557, 559
 chorionic trophoblast, in Mammal, Pig, 535, 574, 575 (See also trophoblast)
 chorionic villi, in Mammal; Cat, 539, 540; Man and Apes, 546, 547, 554, 555, 556, 557
 choroid coat, in Frog, 192; in Chick, 418, 419
 choroid fissure, in Frog, 190, 190, 191, 192; in Chick, 353, 354, 380, 419, 419, 435
 choroid knot, 192
 choroid plexus,
 anterior, in Frog, 178, 179, 180; in Chick, 411, 412; in Pig, 612
 posterior, in Frog, 178, 181; in Chick, 412, 413; in Pig, 613
 chromaffin cells (See cells)
 chromatid, 18-24, 37
 chromatin, 16, 24, 27
 loss in egg, 26, 27
 nucleolus in, in Chick egg, 285; in Frog, 109

- chromonema, 16-21, 24, 25, 27, 28, 37;
in Frog, 109
- chromosomes, 16-38; in Frog, 108, 109;
in Chick, 285
- cicatrices, in *Amphioxus*, 79; in Chick,
282, 287
- ciliary processes, in Chick, 418, 418
- circle of Willis, in Pig, 594, 595
- circulation,
embryonic, in Chick, 457; in Mam-
mal, 457
extra-embryonic, 346, 347
- circulatory changes at birth or hatch-
ing, 454-460; in Cat, 458
- circulatory system, in Frog, 167-168,
210-225; in Chick, 339-347, 377-
382, 401-408, 450-465; in Pig,
585-603, 634-642
- Clark, S. L., 459
- clavicle, in Frog, 254; in Chick, 438
- claw, in Chick, 436
- cleavage (or segmentation) (*See also*
segmentation)
- Clements, L. P., 632
- clitoris, in Pig, 645, 647, 652, 654
- cloaca, in Frog, 105, 208; in Chick,
282, 283, 368, 376, 391, 400, 427,
448, 448, 449, 449, 467; in Pig,
568, 569, 583, 583, 645, 647
- cloacal membrane, in Chick, 368, 448,
449, 449; in Pig, 583 (*See also* anal
plate)
- club-shaped gland, in *Amphioxus*, 93
- clutch of eggs, in Chick, 291, 292
- coelom, 63, 64; in *Amphioxus*, 96, 98;
in Frog, 164, 165, 210; in Teleost,
274; in Chick, 322, 326, 329; in
Mammal, 528; Pig, 569, 575, 578,
579, 633, 634
extra-embryonic, in Mammal, 516,
517; Man, 552
pericardial, in Pig, 569
- coelomic space, in Pig, 528
- Cole, R. K., 289
- collecting ducts or tubules, in Chick,
390, 427, 474; in Pig, 644
- colliculi,
inferior, in Pig, 612
superior, in Pig, 612
- colon, in Pig, 627, 629
ascending, in Pig, 629
descending, in Pig, 629
- columella, in Frog, 196; in Chick, 424;
in Pig, 619
- commissure, in Frog, 180
anterior, in Chick, 383, 411, 412
habenular, in Chick, 412
infundibular, in Chick, 412
pallial, in Chick, 412
posterior, in Chick, 411, 412
spinal, in Chick, 412
trochlearis, in Chick, 411
- common trunk, of pronephros, in Frog,
226
- competence, in induction, in Frog, 141
- conchae, in Pig, 616
- concrecence,
in gastrulation, 61, 61
or convergence, in Teleost, 267
- confused or diffuse stage, in meiosis, 23
- Congdon, E. D., 453
- conjugation, in Protozoa, 48
- Conklin, E. G., 75
- Conrad, R. M., 289
- contraction stage, in meiosis, 17, 18
- convergence, 61, 62; in *Amphioxus*, 90;
in Teleost, 269; in Frog, 127, 128,
134; in Chick, 305, 306
- Copenhaver, W. M., 168, 215, 216
- coprodaeum, in Chick, 448, 448, 449,
449
- copula, in Frog, 251
- copulation path of sperm, 44, 46; in
Frog, 114, 115, 116
- copulation plane, in Frog, 115
- coracoid, in Chick, 438
- cords of Pflüger, in Mammal, 490
- cornea, in Frog, 192; in Chick, 418, 421
- Corner, G. W., 498, 499, 504
- Cornman, I., 113
- cornu, greater and lesser (*See* hyoid)
- corona radiata, in Mammalian follicle,
490, 492, 492
- coronary sinus, in Pig, 640
- corpora quadrigemina, in Pig, 567,
610, 612
- corpora striata, in Chick, 409, 411
- corpus luteum, in Mammal, 494, 495,
495, 496, 497, 499, 500, 502
- cortex,
of gonad, in Chick, 468
of hair, in Pig, 663
- cortical substance of adrenal, in Frog,
232, 233; in Chick, 428, 475
- costal process, in Chick (*see* trans-
verse); in Pig, 655, 656
- cotyledons, in Mammal; Ungulates
(Cow), 536

- Cow,
 implantation in, 536, 537
 placental villi in, 502, 534, 536
 pro-oestral bleeding in, 496
 Cowper's glands, in Mammal, 488
 cranial flexure, in Chick, 322, 333, 349,
 370, 379, 395, 409; in Pig, 562,
 566
 cranial ganglia and nerves, in Frog,
 182-187; in Chick, 352, 353, 415,
 416 (See also ganglia and nerves)
 cranium, in Chick, 440-441; in Pig, 655
 crop, in Chick, 446
 cross-overs, genetic, 21
 mechanisms of, 37, 38
 crown, of tooth, 660, 661, 662
 crown rump axis, in Pig, 563
 crura cerebri, in Frog, 181; in Chick,
 413; in Pig, 612
 cumulus oöphorus, in Mammal, 492
 cushion septa, in Chick, 402, 403, 450,
 451; in Pig, 589, 641
 cuticle, in hair, 663
 cutis layer, in Amphioxus, 100
 cutis plate (See dermatome)
 cystic duct, in Chick (ductus cysticus),
 447; in Pig, 580, 580, 630
 cytoplasm, of egg, 10
- dahlite, in tooth, 660
 Danchakoff, V., 340
Dasyurus,
 allantois in, 530, 532
 implantation in, 532
 yolk-sac in, 530, 532
 yolk-sac placenta in, 530, 532
 decidua, in Mammal; Man, Apes, 559
 basalis (serotina), in Mammal; Man
 and Apes, 552, 554, 555, 557, 559
 capsularis (reflexa), in Mammal; 552,
 557, 559
 compacta, in Mammal; Cat, 539-
 Man and Apes, 558
 spongiosa, in Mammal; Cat, 539;
 Man and Apes, 558
 vera, in Mammals; Man and Apes,
 557, 559
 delamination,
 gastrulation by, 58, 59; in Frog, 133;
 in Chick, 303; in Mammal, 510
 mesoderm separation by, 65, 66; in
 Frog, 134
 dental lamina or ledge, in Pig, 624, 658,
 661
- dental papilla (pulp), in Pig, 623, 658,
 659
 dental sac, in Pig, 623, 651
 dentine, in Pig, 623, 658, 659, 660, 661,
 662
 dermatome (cutis plate), 64, 69; in
 Amphioxus, 99, 100; in Frog, 166,
 166; in Chick, 329, 335, 371, 396,
 397; in Pig, 585
 dermis, in Frog, 209; in Chick, 371, 436;
 in Pig, 585
 Detwiler, S. R., 209, 386
 developmental concepts, 143, 144
 diakinesis, in meiosis, 20, 23, 24, 37
 diaphragm, in Pig, 573, 633, 633, 634
 diaphragmatic ligament, in Pig, 646,
 650, 651
 diaphysis, in Frog, 243, 252, 253, 254;
 in Chick, 439, 440; in Pig, 656, 657
Didelphys (See Opossum)
 diencephalon, in Frog, 179, 180; in
 Chick, 333, 334, 349, 350, 380, 383,
 384, 410; in Pig, 566, 568, 610, 611,
 612
 digestive system, in Pig, 573-584, 622-
 632 (See also alimentary tract)
 digits, in Chick, 439; in Pig, 606, 607,
 657
 dioestrus, 494, 495, 497, 498, 502
 diploid, in meiosis, 17
 diplotene, 18-21, 18, 21, 23, 27, 30
Discoglossus, gastrulation in, 137, 139
 discus proligerus, in Mammalian folli-
 cle, 490, 491
- Dog,
 allantois in, 538
 amniotic cavity in, 538
 egg of, 492, 510
 mesometrium in, 538
 placenta in, pro-oestral bleeding in,
 496
 sex cycle in, 495, 496, 501
 dorsal flexure, in Pig, 562
 dorsal thickening of brain, in Frog, 157
Drosophila, 24, 35, 36
 ductus Botalli, or arteriosus, in Frog,
 249; in Chick, 404, 404, 452, 453,
 453, 456, 457, 459, 460; in Pig, 636,
 643
 ductus choledochus, in Chick, 375, 447;
 in Pig, 630, 631
 ductus cochlearis, or cochlear duct, in
 Chick, 422, 422; in Pig, 618, 618,
 619

- ductus Cuvieri, in Frog, 218, 220, 221, 221; in Chick, 333, 334, 345, 346, 378, 379, 381, 381, 405, 408, 463; in Pig, 589, 596, 597, 598, 599, 638, 639
- ductus reuniens, in Pig, 618, 618
- ductus venosus, in Chick, 345, 346, 347, 381, 405, 406, 408, 457, 465; in Pig, 569, 579, 596, 600, 602, 638
- Dudley, J., 336
- duodenal-jejunal flexure, in Chick, 445, 447
- duodenum, in Frog, 206; in Chick, 399, 445, 445, 446, 447; in Pig, 580, 627, 629
- Du Shane, G. P., 209
- dyads, in *Ascaris*, 23
- ear,
 external, in Pig, 607, 608, 621
 homologies of bones in, in Pig, 620, 622
 inner, in Frog, 192, 193, 194; in Chick, 421, 422, 422, 423; in Pig, 573, 617, 618, 619 (*See also* auditory vesicle)
 middle, in Frog, 195, 196; in Chick, 423, 424; in Pig, 618, 619, 620
 origins of, 67
- Eastlick, H. L., 386
- Echidna*, 531
- ectobronchus, in Chick, 444
- ectoderm, 53; in *Amphioxus*, 88, 92; in Frog, 134; in Teleost, 270, 270; in *Gymnophiona*, 277; in Chick, 302, 306, 307, 308, 309, 309; in Mammal, 516, 517; Pig, 527, 528
- movements during gastrulation, in *Amphibia*, 136, 137, 137, 138, 138, 139
- products of, 67
- Edwards-Jones-Brewer blastocyst, in Mammal, 549, 552
- egg (or ovum), 8, 9, 10; in Frog, 106-120; in Fish, 262, 263; in Chick, 281-290; in Mammal, 489-493
- cylinder, 540
- cytoplasm, reaction to fertilization, 40-43, 41, 42
- fertilized, in *Amphioxus*, 79, 79
- influence compared with that of sperm on early development, 49
- meiosis of, 21, 27, 27, 28, 30, 44, 45; in *Amphioxus*, 77, 78
- numbers spawned, in Frog, 112
- symmetry and orientation, in *Amphioxus*, 79, 80, 81, 82, 83 (*See also* embryonic)
- tooth, in Chick, 476
- ejaculatory duct, in Pig, 646, 648
- Elephant, retention of testes in, 488, 651
- embryology,
 nature of, 2
 relation to genetics, 49
- embryonic axis, determination of, in Chick, 320, 321, 322
- embryonic knob, in Mammal, 513, 525; Rabbit, 514; Pig, 515, 515; Hedgehog, 517, 518; Guinea Pig, 518, 519; Mouse, 520, 521; Primates, 548
- embryonic shield, in Teleost, 268; in Chick, 301
- embryonic symmetry, in *Amphioxus* (*See under* egg), in Frog, 115, 119, 120, 121, 122
- enamel, in Mammal, 623, 658, 659, 661, 662
- formation, in Mammal, 660
- organ, in Mammal, 623, 658, 658, 659, 660, 661
- pulp, in Mammal, 623, 658, 659
- end knob, in sperm, 13
- end piece, in sperm, 12
- endocardial cushion, or cushion septum, in Chick, 339, 402, 403; in Pig, 578, 589, 589, 641
- endocardium, or endothelial lining, in Frog, 168, 189, 210, 211; in Chick, 339, 340, 341, 402, 403; in Pig, 586, 588
- endochondral bone, 243-244
- endocrine glands, effect of on laying, in Chick, 291
- endoderm, 53; in *Amphioxus*, 88, 92; in Frog, 131, 134; in Teleost, 270, 271; in *Gymnophiona*, 277; in Chick, 302, 306, 307, 308, 309, 309, 315, 316, 322; in Mammal, 517; Pig, 521, 527, 528; Primates, 548, 549, 549
- movements during gastrulation in *Amphibia*, 136, 137, 137, 138, 138
- products of, 67
- endolymphatic duct, in Frog, 193, 194; in Chick, 389, 389, 421, 422, 423; in Pig, 573, 617, 618

- endolymphatic outgrowth, in Anura, 194
- endolymphatic sac (sacculus endolymphaticus), in Chick, 421, 422
- endometrium, in Mammal, 489, 494, 500; in Ungulates, 535, 537
- endomixis, 48
- endoplasm, of egg, in Amphioxus, 77
- endosteum, in Frog, 242
- enterocoel, in Amphioxus, 92
- enterocoelic method of mesoderm formation, 63, 64
- enterocoelic pouches, in Amphioxus, 98
- enteron, in Frog, 162, 163
- entobronchus, in Chick, 444
- entrance cone, 41, 41
- entrance path of sperm, 44, 46; in Frog egg, 111, 114, 115
- entrance-path plane, in Frog egg, 115
- entypy, in Mammal, 517
- ependymal cells, in Frog, 181, 182; in Chick, 351, 384; in Pig, 567, 570, 613, 614
- epiblast, 54; in Amphioxus, 88, 89; in Frog, 132, 134; in Teleost, 265, 265; in Gymnophiona, 273; in Chick, 302, 305, 308, 309; in Mammal, 510; Pig, 515, 515
- epiboly, in gastrulation, 60, 60; in Amphioxus, 90; in Frog, 127, 131, 134; in Teleost, 277; in Gymnophiona, 277; in Chick, 318, 319, 361
- epicardium, in Pig, 508, 510, 512, 588
- epidermis, in Amphioxus, 99; in Frog, 125; in Chick, 396
 - source of, 67
- epididymis, in Chick, 472, 473; in Pig, 646, 647, 647, 651
 - appendix to, 647, 649
- epiglottis, in Pig, 627
- epiphyseal cartilage, in Mammal, 243
- epiphyseal plate, in Mammal, 657
- epiphysis, in Frog, 177, 177, 178, 180; in Chick, 350, 379, 383, 410, 411, 412, 435; in Pig, 612
 - of bone, in Frog, 253, 254; in Chick, 439, 439, 440; in Mammal, 656, 657
- epiploic foramen, in Pig, 626, 629
- epithelioid bodies, in Frog, 202, 204, 205, 205, 217
- epithelial vestiges, in Chick, 442, 443; in Pig, 624, 625, 625 (See parathyroids, postbranchial bodies, thymus, and tonsils)
- epithelium,
 - of oviduct, 489
 - of uterus, 497
- epoöphoron, in Chick, 473; in Pig, 649, 653
- equational meiotic division, 19, 21, 22, 24, 25
- erectile muscles of hair, in Pig, 664, 664
- Erythrocytes (See blood corpuscles)
- esophagus, in Frog, 207; in Chick, 372, 373, 399, 445; in Pig, 568, 569, 578, 579, 627, 627
- Etkin, W., 174
- Eustachian tube, in Frog, 195; in Chick, 423; in Pig, 573, 618, 620
- Everett, N. B., 6, 7
- evocation, in Frog, 141
- excretory system, in Frog, 225-233; in Chick, 355-357, 390, 391, 427-428, 466-468, 468; in Pig, 605, 643, 644
- exocoelom, in Mammal, Pig, 523; Primates, 546, 547, 549
- exoplasm, of egg, in Amphioxus, 77
- external appearance, in Chick, at five days, 433-436; in Pig, at 10 mm., 562-666, later, 606-609
- external auditory meatus, in Chick, 424, 433
- external limiting membrane of nerve cord, in Pig, 570
- eye, in Frog, 189; in Chick, 353, 354, 379, 388, 417-421, 435; in Pig, 564, 617
 - lid, in Chick, 435; in Pig, 609
 - sources of, 67
 - transplantation to tail, in Frog, 174, 175
- face, in Chick, 433, 434, 435; in Pig, 566, 608, 608, 609
- falciform ligament, in Pig, 626, 631, 633
- Fallopian tubes, in Mammal, 489 (See also oviduct)
- false amniotic cavity, in Mammal, Guinea Pig, 519; Mouse, 520, 521
- Farris, E. J., 507
- fasciae, in Amphioxus, 100
- fat bodies, in Frog, 105, 105, 238
- feather, source of, 67
- feather barbs, in Chick, 436
- feather down, in Chick, 436
- feather germs, 433, 435
- feather pulp, 435, 436
- feather quill, 436

- feather rachis, 436
 femur, in Pig, 650
 fenestra ovalis, in Frog, 196; in Chick, 423, 424; in Pig, 618, 619, 620
 fenestra rotunda, in Chick, 423; in Pig, 618, 619
 fertilization, 39-48; in Amphioxus, 79; in Frog, 113-116; in Chick, 287; in Mammal, 506
 consequences of, 47-48
 effect on of numbers and motility of sperm, 507, 508
 nature of, 2
 fertilization membrane, 40; in Amphioxus, 77, 79, 80
 fertilizin theory, 43
 fetal circulation, changes in at birth, 643 (*See also* circulation)
 fibers of Sharpey, 247
 fibroblasts, 240, 241, 241, 242, 243, 244
 fibula, in Pig, 658
 Figge, F. H. J., 174
 Finnegan, C. V., 216
 Firket, J., 470
 flagellum, of sperm, 12, 12
 flexures and torsions, in Chick, 332, 333, 370, 395, 409; in Pig, 562, 606
 follicle, of egg, 4, 5; in Frog, 107, 108, 237; in Chick, 281, 282, 283, 472
 follicle, Graafian, in Mammal, 490, 491, 493, 495, 495, 498, 500, 501
 follicular cavity, in Mammal, 491
 foramen caecum, in Man, 627
 foramen ovale, in Mammal, closure at birth, 450, 459; in Pig, 641, 642, 643
 foramina of Monro, in Frog, 179; in Chick, 350, 411; in Pig, 611
 fore-brain (*See* prosencephalon)
 fore-gut, in Frog, 162; in Chick, 306, 320, 323, 324, 328, 335-337, 371-375, 398, 399, 442-446; in Pig, 574, 576-580, 625-628
 formative materials of egg, in Amphioxus, 79, 82, 83
 distribution of in Frog and other Amphibia (*See* map)
 fovea, in Frog egg, 109, 110
 Franklin, K. J., 458
 Fraps, R. M., 292
 Fraser, R. C., 303, 370
 Friedman, test for pregnancy, 503
 Frog,
 early development; external, 147-155; internal, 155-169
 later development, 169-254
 reasons for study of, 104
 stages, external, 170
 frontal process (*See* naso-frontal)
Fundulus, egg of, 263
 fundus, of eye, in Chick, 388, 417
 fusion, of egg and sperm nuclei, in Amphioxus, 80; in Frog, 114
 gall bladder, in Frog, 178, 206; in Chick, 374, 375, 447; in Pig, 580, 580, 582, 630
 gametes, 3
 gamones, 39
 ganglia or ganglion, cranial,
 acustico-facialis, VII, VIII, in Frog, 185, 186, 187; in Chick, 352, 353, 379, 387, 388, 415; in Pig, 568, 570 (*See also* geniculate)
 ciliary, in Chick, 416
 glossopharyngeal, IX, in Frog, 186; in Chick, 352, 353, 353, 379, 415; in Pig, 570, 571, 571
 jugulare, X, in Chick, 415, 416; in Pig, 568, 571, 615
 neunogastric, X, in Chick, 415
 nodosum, X, in Chick, 416; in Pig, 568, 571, 615
 petrosal, IX, in Pig, 568, 571, 571
 trigeminal or Gasserian, V, in Frog, 185; in Chick, 334, 352, 353, 387, 415; in Pig, 568, 570
 vagus, in Frog, 186; in Chick, 352, 353
 ganglia or ganglion, spinal, in Frog, 187, 187, 188; in Chick, 329, 351, 385, 396; in Pig, 568, 569, 569, 570, 571, 572
 accessory or Foriep's, in Pig, 568, 571, 571, 615
 sympathetic, in Frog, 189; in Chick, 387, 414, 414; in Pig, 572, 616
 gastro-hepatic ligament, in Chick, 375; in Pig, 630
 gastrula, of Amphioxus, 88; of Triton, 137; of Teleost, 269; of Gymnophiona, 277
 gastrular cleavage, in Frog, 134
 gastrular movements, in Frog, 136, 137, 137, 138, 138
 gastrulation, in Amphioxus, 87-91; in Frog, 126-134, 126, 130, 131; in Teleosts, 264-269; in Gymnophiona, 273-276; in Chick, 300-316,

- 320; in Mammal, 508, 511-513, 527
 general discussion of, 50, 53-63, 15, 57, 58, 60, 61, 62
 Geinitz, B., 140
 genes, 27, 37
 geniculate, VII cranial ganglion, in Mammal, Pig, 570
 genital cavity, in Frog, 236, 236, 237, 237
 genital eminence, in Pig, 653
 genital fold, in Pig, 652, 653
 genital ridge, in Pig, 582
 genital ridges, 3; in Frog, 235
 genital swelling, in Pig, 652, 653, 654
 genital tubercle, 645, 652, 653
 genitalia, in Pig, 652, 653-654
 germ cells, 3; in Frog, 236, 236, 237, 237; in Chick, 469, 469, 470, 472
 germ layers, inversion of, in Mammal, 521, 522
 germ ring, 55, 62, 63; in Amphioxus, 91, 97; in Frog, 128; in Teleost, 270; in Gymnophiona, 274, 276
 germ wall, in Chick, 294, 297, 301, 308, 310, 317, 320, 322
 germinal cells, in Chick, 351; in Pig, 567
 germinal disc, 282, 284
 germinal epithelium, 3, 4; in Chick, 390, 469, 469, 470, 470, 471, 471; in Mammal, 490, 490, 491
 germinal vesicle, 9; in Chick, 281, 287
 Gilbert, M. S., 502
 gill chamber (opercular), in Frog, 202, 203
 gill circulation, in Frog, 202, 216, 217
 gill plate, in Frog, 148, 150, 151
 gill rakers, in Frog, 203, 204
 gills, in Frog, 170, 171, 202, 203, 203
 gizzard, in Chick, 445, 446, 447
 glandular part of oviduct, in Chick (magnum), 475
 glia cells, in Frog, 181; in Chick, 351, 385
 glomerulus, in Frog, 232; in Chick, 390, 391, 474; in Pig, 579, 604, 644
 glomus, in Frog, 226, 227, 228, 229; in Chick, 356
 glottis, in Frog, 178, 206; in Chick, 398, 443, 445; in Pig, 627
 glycogen tissue, in Mammal, 543, 544, 545
 Godwin, M. C., 625
 Goerttler, K., 137
 Golgi apparatus, in Frog egg, 109
 Goldsmith, J. B., 469
 gonad or gonads, 3; in Amphioxus, 76, 76; in Frog, 234-240; in Chick, 427, 445, 468, 472, 474; in Pig, 605, 645
 gonoducts, in Frog, 233; in Chick, 428
 Goss, C. M., 216
 granulosa, in Chick, 281, 283
 gray crescent, in Frog, 117, 118
 inducing material, in Frog, 138, 140
 plane, in Frog, 116, 118
 gray matter, of nerve cord, in Frog, 181, 182; in Chick, 385; in Pig, 614
 Grier, N., 113
 Gruenwald, P., 376, 490
 gubernaculum, in Pig, 646, 650, 651
 Guinea Pig,
 amnion formation in, 518, 519, 519
 blastocyst in, 518, 519
 circulation changes in at birth, 459
 embryonic knob in, 518, 519
 endoderm in, 513
 inversion of germ layers in, 521
 sex cycle in, 496, 500, 501, 507
 survival of egg in, 508
 survival of sperm in, 508
 yolk-sac in, 519, 545
 gum, in Pig, 658
 gut, in Amphioxus, 92, 93; in Frog, 162, 163, 200-208; in Teleost, 274; in Chick, 335-338, 371-377, 398-401, 442-449; in Pig, 568, 582, 583
 diverticulum, in Amphioxus, 93, 100, 101
 folding-off of, in Pig, 574, 574, 575, 575
 formation of, in Mammal, 513; Pig, 573
 loop, in Pig, 569, 581, 582
 post anal or cloacal, in Chick, 375, 376, 377; in Pig, 568, 583
 gynagamones, 39
 hair, in Mammal, 662-664
 follicle, 663
 germ, 663
 matrix, 662
 papilla, 662, 663, 664, 664
 root, 663
 shaft, 664
 sources of, 67
 half embryo, in Frog, 121

- Hamburger, V., 386
 Hammond, W. S., 387, 416, 443
 haploid, chromosome number, 17, 18, 22, 24
 hard palate, in Pig, 622
 harelip, 609
 Hargitt, G. T., 491
 Hartman, G. T., 493, 498
 Harvey, 455
 hatching, of Chick, 475, 476
 Hatschek, B., 75
 Haversian canal, 244, 245
 Haversian system, 245, 245, 656
 head and neck region, in Pig, 607-609
 head of sperm, 11, 12
 head fold, in Chick, 320, 321, 323, 324, 324
 head process, in Chick, 305, 306, 307, 309, 309, 310, 311
 heart,
 changes in at hatching or birth, 454-460
 development of, in Frog, 167-168, 210-213; in Chick, 339, 341, 342, 343, 344, 401-403, 450, 451; in Pig, 565, 574, 586, 587, 588, 588, 589, 589, 633, 641, 643
 initiation of beat in, in Frog, 214, 215; in Chick, 342, 343
 muscle, in Frog, 167
 potentiality of parts, in Frog, 168
 heat (*See* oestrus)
 Hedgehog,
 amnion in, 518
 blastopore in, 526, 526
 yolk-sac in, 518
 Helff, O. M., 174, 176, 196
 Hemichromis, 266, 268
 Hensen's node or knot, in Chick, 305, 305, 306, 307, 308, 309, 310; in Mammal, Pig, 523, 524, 525, 526 (*See also* primitive pit)
 hepatic ducts, in Pig, 580, 580 (*See also* bile)
 hepatic portal system, in Frog, 221; in Chick, 457, 461, 462; in Pig, 597, 600, 638
 Hertig, A. T., 553
 Hertig-Rock blastocyst, 552
 Hertwig, O., 48
 heterotypic chromosomes, 18, 20, 24, 37
 Hibbard, H., 109
 Hilleman, H. H., 371
 hind-brain (*See* rhombencephalon)
 hind-gut, in Frog, 163, 207; in Chick, 337, 338, 375-377, 400, 401; in Pig, 574, 576, 581
 hind-limb, buds, in Chick, 404
 Hinsey, J. C., 504
 Hisaw, F. L., 500, 501
 Holtfreter, J., 126, 143, 144
 Holley, E., 174
 Holtzer, H., 247
 homolecithal eggs, 10
 homotypical chromosomes, 24
 hoofs, 664
 Hook, S. J., 503
 horns, 67, 664
 human embryo, 558, 559
 humerus, in Chick, 400
 Humphrey, R. R., 239
 Hunt, E. A., 386
 Hunt, T. E., 300, 309
 Huth, T., 174
 hyoid arch, in Frog, 153, 160; in Chick, 336; in Pig, 565, 566, 576
 hyoid cornu or horn, in Frog, 251, 253; in Chick, 441; in Pig, 624
 hyomandibular cleft, in Frog, 150, 151; in Pig, 564, 565, 576
 hyomandibular pouch, in Frog, 160, 162, 195, 201, 201; in Chick, 336, 398; in Pig, 573, 577, 619
 hypoblast, 54; in Amphioxus, 88, 89; in Frog, 132, 133, 134; in Chick, 302, 302, 303, 304, 309; in Teleost, 265, 265; in Gymnophiona, 273; in Mammal, 512; Pig, 508, 510, 511, 513, 515, 515
 hypobranchial apparatus, in Frog, 250, 251, 251, 253
 hypobranchial plate, in Frog, 203
 hypochordal rod, in Frog, 163, 164
Hypogophis, gastrulation in, 275, 276, 277
 hypophysis (*See* pituitary)
 Ichthyophis, gastrulation in, 276
 idiozome, 11
 ilium, in Chick, 438; in Pig, 627, 629, 657
 illumination, effect on laying, in Chick, 292
 implantation, 513; in Ungulates, Pig, 535; in Carnivores, 539-540; in Rodents, 540-543; in Primates, 550-551; Man and Apes, 553-558
 inducing substance, 142-143

- induction or evocation,
 - general principle, 141, 143-144
 - special cases, 140, 141, 142, 143, 161, 190
- infiltration,
 - gastrulation by, 58, 59; in Chick, 303
 - mesoderm origin by, in Chick, 309
- infundibulum,
 - of brain, in Frog, 157, 158, 177, 178, 178; in Chick, 348, 349, 349, 371, 372, 384, 410, 411, 412; in Pig, 567, 611
 - of oviduct, in Frog, 107; in Chick (also ostium), 282, 283, 475; in Mammal, 489; in Pig, 649
- ingression, in Frog, 133
- inguinal canal, in Pig, 646, 651
- inguinal ligament (in adult Poupart's), 646, 647, 650, 653
- inner cell mass, in mammals, 508, 509, 510, 510, 512, 513
- inner ear (See membranous labyrinth)
- inner tubule of mesonephros, in Frog, 230, 231
- inner zone, of nephrogenous tissue, in Chick, 467, 468, 468, 474
- Insectivores, amnion formation in, 514
- interatrial foramen or foramina, in Chick, 455, 457, 459; in Pig (primum), 588, 589, 589, 593, 642, (secundum), 588, 589, 589, 641, 641, 642
- interatrial septum, in Frog, 213; in Chick, 377, 402, 403, 450, 457; in Pig (primum), 588, 588, 641, 641, 642, (secundum), 589, 589, 641, 641, 642
- intermediate cell mass, 69
- intermenstrual bleeding, in Man, 498
- internal limiting membrane,
 - of eye, in Frog, 192; in Chick, 417
 - of neural tube, in Pig, 567, 570
- internasal septum, in Chick, 441; in Pig, 616, 621
- interorbital septum, in Chick, 441
- intersomitic fissure, in Chick, 396
- interventricular foramen, in Pig, 589, 593
- interventricular groove, in Chick, 402
- interventricular septum, in Chick, 402, 403, 450, 451; in Pig, 578, 589, 589, 642
- intervertebral fissure, in Chick, 396
- intestinal caecae, or caecal processes, in Chick, 400, 445, 447, 448
- intestinal portal,
 - anterior, in Chick, 323, 324, 339, 405; in Pig, 574
 - posterior, in Chick, 337, 338, 376, 377; in Pig, 574
- intestine, in Frog, 178, during metamorphosis, 171, 173; in Chick, 445, 446, 447, 448; in Pig, 581, 627, 628, 629
- invagination,
 - gastrulation by, 54, 55, 57; in Amphioxus, 87, 88; in Frog, 131, 132, 134; in Chick, 303, 305, 309
 - mesoderm separation by, 66, 67
- involution,
 - gastrulation by, 56, 56, 57; in Amphioxus, 87, 88; in Frog, 131, 133, 134; in Teleost, 264, 265; in Gymnophiona, 277; in Chick, 302, 302
 - mesoderm separation by, in Chick, 302, 308, 309; in Pig, 527
- iris, in Frog, 192; in Chick, 418, 418
- ischium, in Chick, 438; in Pig, 657
- islets of Langerhans, in Pig, 631
- isthmus,
 - of brain, in Chick, 379, 384, 384, 410; in Pig, 567, 570
 - of oviduct, in Chick, 282, 283, 289
- iter (See aqueduct of Sylvius)
- Jacobson, W., 303
- Jacobson's organ, in Frog, 197, 199
- Janes, R. G., 207
- jaw, in Chick, 434; in Pig, 608
- jejunum, in Pig, 627 (See also small intestine)
- jelly, of egg, in Frog, 111, 112
 - effect on temperature, 113
- Jennings, H. B., 48
- Jones, D. S., 352, 387, 415
- Jordan, E. S., 508
- Kaan, H. W., 195
- karyosome, 28
- Kellogg, H. B., 458
- Kemp, N. E., 109
- Kennedy, J. A., 458
- kidney, in Frog, 105, 229-232, 230
 - head, in Frog, 155
- Klapper, C. E., 624
- Knouff, R. A., 185
- Kollros, J. J., 181

- Kuo, Z. Y., 476
 Kupffer's vesicle, in Teleost, 265, 269, 270, 314
- labio-dental, ledge or lamina, in Pig, 624, 658
 labio-gingival groove, in Pig, 623, 624, 658
 labium majora, in Pig, 647, 652, 654
 labium minora, in Pig, 647, 652, 654
 lachrymal duct, in Pig, 609
 lachrymal groove, in Chick, 434, 435; in Pig, 564, 607, 608, 609
 lacunae in placental trophoderm, of Hedgehog, 518; of Guinea Pig, 519; of Rabbit, 543, 545; of Mouse, 544; of Man and Apes, 554, 555, 556
 lagena, in Frog, 193, 195; in Mammal, 618, 618
 lamina,
 post optica, in Pig, 611
 terminalis, in Chick, 350; in Pig, 569
 laryngotracheal groove, in Chick, 372, 373, 373; in Pig, 579
 larynx, in Frog, 206; in Chick, 337, 398, 443; in Pig, 627
 latebra and neck of, in Chick, 281, 284, 286
 lateral closing folds, in Chick, 465
 lateral limiting sulcus, in Chick, 329
 lateral line organs (ramus lateralis), in Frog, 198, 199
 lateral nasal process, in Chick, 433, 435, 435
 lateral neural ridges or folds, in Frog, 136, 148
 lateral plate, 64, 68; in Amphioxus, 99; in Frog, 165; in Chick, 324, 333, 395
 lateral rotation, in Chick, 333, 395
 lateral torsion, in Pig, 563
 lateral ventricles, of brain, in Frog, 177; in Pig, 511
 latero-bronchi, in Chick, 444
 laying periodicity in Hens, 290-292
 legs, in Frog, 172
 Lemurs, sex cycle in, 497
 lens of eye, in Frog, 190, 190, 191, 192; in Chick, 354, 354, 388, 417, 418, 420, 435; in Pig, 573, 576
 lenticular zone, in Chick, 417
 leptotene, stage in meiosis, 16, 18, 20
 Levi-Montalcini, R., 416
- Lewis, W. H., 190
 lids, of eye, in Chick, 418, 421
 Liedke, K. B., 190
 Lillie, F. R., 376, 378, 416
 limb or limbs, in Chick, 438, 439; in Pig, 606
 buds, in Chick, 370, 395, 433; in Pig, 564, 565, 578, 579, 583
 determination of axes in, in Frog, 172; in Chick, 333, 334
 limiting sulci, in Chick, 362
 Lindeman, V. F., 174
 lips, in Frog, 200
 liquor folliculi, in Mammal, 491
 liver, in Frog, 206; in Chick, 337, 374, 374, 399, 405, 445, 446, 465; in Pig, 565, 568, 569, 578, 579, 580, 581, 581, 582, 626, 630, 633
 evagination, in Frog, 157, 162, 165, 177
 source of, 67
 lumbo-sacral flexure, in Pig, 562
 lungs, in Frog, 206; in Chick, 372, 373, 380, 381, 384, 443, 444; in Pig, 568, 569, 577, 578, 632, 632
 homologues, 337
 Lygeus bicrucis,
 meiosis in, 30
 sex-chromosomes in, 30, 34, 34, 35, 36
 lymphatics, in Frog, 225
- magma reticulare, in Man, 548, 549, 549
 magnum, in Chick oviduct, 282, 283, 289
 main piece, of sperm, 12
 malleus, in Mammalian ear, 618, 619, 620, 655
 Malpighi, 280
 Malpighian body, in Frog, 232; in Chick, 357, 427, 436, 471, 475
 Malpighian layer, in Chick, 436
 Mammal,
 early stages of, 506-560
 embryological significance, 486, 487
 gastrulation in, 58, 59
 sexual cycle in, 493-504
- Man,
 allantois in, 546, 547
 amnion in, 547
 amniotic cavity in, 546, 547, 548, 552
 blastocyst in, 548, 548, 549, 549
 blastoderm in, 548, 548, 549, 549
 Heuser's membrane in, 548, 549

- implantation in, 553-558
- ovary in, 488
- sexual cycle in, 497, 498, 503
- sperm travel in, 507
- uterus in, 548
- yolk-sac in, 546, 547, 549, 549
- mandibular arch, in Frog, 151, 160, 163, 201, 201; in Chick, 334, 336, 434, 435; in Pig, 564, 565, 566, 576, 608, 608
- mandibular cartilage, in Pig, 621
- mandibular nerve, in Frog, 185
- mandibular ridges, in Frog, 200
- Mangold, O., 139
- mantle layer, in neural tube, Pig, 567, 568, 570, 613, 614
- map of formative materials in pregastular stages, in Amphibia, 138, 139; in Teleost, 271, 272, 273; in Chick, primitive streak blastoderm, 311, 312
- margin of overgrowth, in Chick, 322
- marginal layer of nerve cord, in Pig, 568
- Markee, J. F., 504
- marrow, 242, 246; in Frog, 252; in Chick, 439, 440
- Marsupials,
 - allantois in, 530, 532
 - amnion in, 530
 - implantation in, 530, 532, 533
 - placenta in, 530, 532, 533
 - pouch of, 531
 - yolk-sac in, 530, 531, 532
- Martin-Falkiner blastocyst, 550, 552
- Marx, A., 140, 141
- massa intermedia, in Pig, 612
- maturation or meiosis, in Amphioxus, 78, 80; in Frog, 110, 114; in Chick, 287; in Mammal, 505, 509
- maxillae, in Pig, 622
- maxillary nerve, in Frog, 185; in Chick, 415, in Pig, 614
- maxillary process, in Frog, 249; in Chick, 434, 435; in Pig, 564, 565, 566, 576, 607, 608, 608, 609, 622
- McClendon, J. F., 121
- McEwen, R. S., 266
- McKeehan, M. S., 354
- mediastinum, in Pig, 632, 634
- medulla,
 - of brain, in Frog, 181; in Chick, 413; in Pig, 610, 613
 - of hair, 663
- medullary or neural folds, in Amphioxus, 91, 92, 94; in Frog, 136, 143, 152, 154; in Chick, 306, 323, 325, 327; in Pig, 562, 565
- medullary or neural groove, 64, 70; in Frog, 136, 143, 152; in Chick, 327; in Pig, 562, 565
- medullary or neural plate, 64, 70; in Amphioxus, 88, 91, 92; in Frog (or Amphibia), 134, 135, 136, 138, 139, 139, 143, 154; in Chick, 306, 308, 310, 326; in Pig, 565, 575
- medullary substance,
 - of adrenal, in Frog, 232, 233; in Chick, 428, 475; in Pig, 644, 615
 - of gonad, 4
- meiosis, 16-26, 27, 28, 34
 - comparison of, in egg and sperm, 28, 29
 - significance of, 37
- meiotic divisions, 19, 21, 22, 23, 24, 25, 25, 28, 29, 30, 31-36, 37; in Amphioxus, 78, 80 (See also maturation)
- membrana granulosa, in Mammal, 490, 491
- membrana propria, in Chick, 423; in Pig, 618, 619
- membrane or membranes, of egg, 11
 - undulatory in sperm, 13
 - vitelline, 11, in Frog, 109; in Amphioxus, 77, 78, 79, 80; in Chick, 284, 286; in Mammal, 493
- membranous labyrinth, in Frog, 192, 193; in Chick, 421-423, 422; in Mammal, 617, 618, 619
- menstrual cycle, 495, 497-501
- menstruation (See menstrual cycle)
- merocytes, in Teleosts, 264; in Chick, 287, 294
- mesectoderm, 54
- mesencephalon (mid-brain), in Frog, 156, 157, 177, 178, 180, 181; in Chick, 333, 334, 348, 348, 349, 350, 373, 383, 384, 410, 412, 413; in Pig, 567, 568, 569, 570, 571, 612
- mesenchyme, in Frog, 135; in Chick eye, 419, 419, 420
- mesentery, 64, 65; in Frog, 210, 235; in Chick, 337, 338, 339; in Pig (dorsal and ventral), 626, 628, 629, 630
- mesentoderm, 54
- mesoblast, in Chick, 306, 307; in Pig, 548, 549 (See also mesoderm)

- mesocardium or mesocardia, in Frog, 211, 211
 dorsal, in Chick, 341; in Pig, 588, 634
 lateral, in Chick, 345, 381, 465, 466
 ventral, in Chick, 340, 341; in Pig, 588
- mesoderm, 53, 63-67; in Amphioxus, 92, 96, 97; in Frog, 131, 134, 135, 136, 137, 137, 138, 138; in Teleost, 269-272, 271; in Gymnophiona, 277, 277; in Chick, 302, 306, 307, 308, 309, 316, 317; in Mammal, 515, 516, 517, 527, 527, 575, 575
 allantoic, in Mammal, 540, 548
 chorionic, in Mammal, 533, 537, 539, 540, 554, 555, 556
 intermediate, in Pig, 585
 peristomial, in Amphioxus, 98; in Frog, 130
 products of, 67
 somatic or parietal (somatopleure), 63, 64, 65; in Amphioxus, 99; in Frog, 164, 165, 165; in Teleost, 274; in Chick, 322, 326, 397; in Mammal, 527, 528; Pig, 585
 splanchnic or visceral (splanchnopleure), 64, 65; in Amphioxus, 99; in Frog, 164, 165, 165; in Teleost, 274; in Chick, 322, 326; in Mammal, 527, 528, 585, 586
- mesogastrium, in Pig, 626, 628, 632, 633
- mesometric side of uterus, 541
- mesonephric duct, in Frog (*see* Wolffian); in Chick (*see* Wolffian); in Pig, 568, 604, 605, 645, 648, 649 (*See also* Wolffian)
- mesonephric tubules, in Frog, 231; in Chick, 357, 390, 391, 427, 466, 472; in Pig, 604
- mesonephric vesicles (units), in Frog, 229, 229, 230, 231
- mesonephros or Wolffian body, in Frog, 229-232, 229; in Chick, 355, 357, 406, 426, 427, 445, 466, 467, 467, 468, 472, 473; in Pig, 565, 568, 569, 579, 585, 604, 605, 643, 644, 645
- mesororium, in Frog, 104, 236; in Chick, 281
- mesovarium, in Frog, 236; in Chick, 282
- metamorphosis, normal and experimental, in Amphibia, 173-176
- metanephric duct (*See* ureter)
- metanephric tubules, in Chick, 468
- metanephros, in Chick, 355, 427, 466, 467, 474; in Pig, 568, 604, 644, 645, 646
- metatarsals, in Chick, 439
- metencephalon, in Frog, 180, 181; in Chick, 333, 334, 349, 351, 384, 384, 410, 413; in Pig, 566, 568, 569, 571, 612, 613
- micropyle, 39; in fish egg, 268
- mid-brain (*See* mesencephalon)
- middle ear (tubo-tympanic cavity), in Frog, 195; in Chick, 423; in Pig, 619
- middle piece of sperm, 11, 12, 13
- mid-gut, in Frog, 162, 163, 207; in Chick, 337, 375, 400, 446-448; in Pig, 581
- milk ridge and nipples, in Pig, 607
- Miller blastocyst, in Man, 548, 552
- Miller and Wiltberg, pregnancy test, 503
- Mole,
 blastopore in, 526
 maturation in egg of, 505, 506
 neurenteric canal in, 526
 primitive streak in, 526
- Money, W. L., 507
- Monkeys and Tarsius,
 implantation in, 550, 551, 551
 sexual cycle in, 497, 499
- monoestrus sex cycle, 496
- monospermy, 39
- Monotremes,
 allantois in, 530, 531
 annion in, 530
 yolk-sac in, 509, 530, 531
- Morgan, T. H., 121, 321
- morula in Mammal, 509, 510
- Mouse,
 allantois in, 542
 amnion in, 520, 521
 egg size, 492
 fertilization in, 508
 implantation in, 540-542, 541
 inversion of germ layers in, 521
 mesometrium, 541
 placenta in, 541, 542, 543, 544, 545
 sex cycle in, 496
 umbilical cord in, 541
 yolk-sac in, 540, 541, 543, 545
- mouth, in Amphioxus, 93; in Frog,

- 171, 200; in Chick, 433; in Pig, 609, 622
- mucosa, of uterus, 494, 495, 496, 497, 502
- mucous gland ("oral sucker"), in Frog, 150, 151, 161, 165, 170
- mucous layer of oviduct, in Mammal, 489
- Müllerian duct (*See* oviduct)
- Munro, S. F., 281
- Murray, P. D. F., 371
- muscle, in Chick, 397
- fibrillae, 209
- of oviduct, in Mammal, 489
- source of, 67
- myelencephalon, in Frog, 180; in Chick, 333, 334, 349, 351, 384, 384, 410, 411, 413; in Pig, 567, 568, 569, 612, 613
- myelin substance or sheath, 614, 615
- myocardium, in Frog, 211, 211; in Chick, 339, 340, 341, 377, 378
- myocoel, 64; in Amphioxus, 99, 100; in Frog, 166; in Chick, 335; in Pig, 585
- myotome, 64, 69; in Amphioxus, 99, 99; in Frog, 150, 155, 166, 166, 209; in Chick, 329, 335, 371, 397; in Pig, 585
- nails, 67, 664
- nares,
- external, in Frog, 199; in Chick, 434, 435; in Pig, 608, 609
- internal, in Chick, 434; in Pig, 609, 621, 622, 623
- nasal bridge, in Pig, 608
- nasal cartilage, in Pig, 621
- nasal cavities, in Frog, 199
- nasal chamber, in Pig, 621
- nasal pit (*See* olfactory)
- nasal septum, in Chick (*see* internasal); in Pig, 608, 621
- nasal sinus, in Pig, 616
- naso-frontal process, in Chick, 433, 435; in Pig, 564, 566, 607, 608, 608, 609
- naso-lachrymal groove, in Chick and Pig (*See* lachrymal)
- naso-lateral process, in Chick (*see* lateral nasal); in Pig, 564, 566, 607, 608, 609
- naso-medial process, in Pig, 564, 566, 607, 608, 608, 609, 622
- naso-turbinals, in Pig, 616
- neck of sperm, 12, 12
- Needham, J., 143
- neopallium, in Pig, 611
- nephrocoel, 64, 69
- nephrogenous tissue, in Chick, 329, 390, 467, 467, 474
- nephrostome, in Frog, 201, 226, 226, 228, 229, 230, 231; in Chick, 356
- nephrotome, 69; in Frog, 167, 229; in Chick, 326, 391; in Pig, 585
- nerve or nerves,
- afferent, in Frog, 182; in Chick, 386
- axones, 188, 192; in Chick, 385
- cord, 64, 70; in Frog, 181, 182, 182; in Teleost, 274 (*See also* neural tube)
- efferent, in Frog, 182, 187; in Chick, 386
- mixed, in Frog, 185
- plexuses, in Pig, 572
- nerve or nerves, cranial,
- abducent or VI, in Frog, 187; in Chick, 416; in Pig, 568, 570, 614, 615
- auditory or VIII, in Frog, 185, 186, 187; in Chick, 415; in Pig, 570, 614, 615
- facial or VII, in Frog, 186; in Chick, 387, 415; in Pig, 568, 570, 614
- glossopharyngeal or IX, in Frog, 186; in Chick, 415; in Pig, 571, 615
- hyoid, branch of VII, in Frog, 186
- hypoglossal or XII, in Chick, 416; in Pig, 568, 571, 615
- mandibular and maxillary (also maxillo-mandibular), branch of V, in Frog, 185; in Chick, 379, 415; in Pig, 568, 570, 614
- neural placode (*See* placode)
- oculo-motor or III, in Frog, 187; in Chick, 373, 387, 388, 416; in Pig, 568, 570, 614
- Olfactory or I, in Frog, 185; in Chick, 411, 420, 426; in Pig, 569, 616
- ophthalmic, branch of V, in Frog, 185; in Chick, 379, 415; in Pig, 568, 570, 614
- optic or II, in Frog, 192; in Chick, 417, 418; in Pig, 569, 617
- palatine, branch of V, in Frog, 186
- spinal accessory, XI, in Chick, 416; in Pig, 568, 571, 615

- trigeminal, or Vth, in Frog, 185; in Chick, 387, 415; in Pig, 570, 674
- trochlearis, or IV, in Frog, 187; in Chick, 412, 416; in Pig, 568, 570, 614
- vagus, or X, in Frog, 186; in Chick, 416; in Pig, 568, 578, 615
- nerves, spinal,
 autonomic (sympathetic and/or parasympathetic), in Frog, 187, 189; in Chick, 385, 387, 413, 414, 414, 415; in Pig, 572, 573, 616
 somatic, in Frog, 187, 187, 188; in Chick, 385, 413, 414, 415, 416; in Pig, 570, 572, 615
- nervous layer, in Frog, 125
- nervous system and sense organs, 67;
 in Frog, 155-161, 177-199; in Chick, 326-330, 348-355, 383-390, 409-426; in Pig, 565-573, 610-620
- neural arches, in Frog, 247; in Chick, 397; in Pig, 654
- neural canal, lumen or neurocoel, 64, 70; in Amphioxus, 92, 93, 94; in Frog, 155, 157; in Chick, 327; in Pig, 570
- neural crests, in Frog, 154, 155, 159, 165, 183, 184, 185; in Chick, 329, 330, 351; in Pig, 567, 569
- neural folds, in Amphioxus, 91, 92; in Frog, 147, 148, 152, 154; in Chick, 325; in Pig, 562, 565
- neural groove, in Frog, 136, 147, 148, 154; in Chick, 321; in Pig, 524, 562, 565
- neural plate, in Amphioxus, 91, 92 (See also medullary)
- neural tube, in Amphioxus, 92, 94; in Frog, 147, 148, 149, 155, 157; in Chick, 327, 328; in Pig, 566, 569, 570, 571, 613
- neurenteric canal, 70; in Amphioxus, 93, 94; in Frog, 152, 154, 157, 161; in Gymnophiona, 275; in Chick, 315; in Mammal, 526, 529, 546, 547
- neurilemma, in Pig, 615
- neuroblasts, in Frog, 181, 192; in Chick, 385, 385, 388, 414, 415, 425; in Pig, 568, 569, 613, 614
- neurones, in Chick, 385; in Pig, 572
- neuropore, 70; in Frog, 156, 157; in Chick, 327
- nictitating membrane, in Chick, 418, 435
- nodes of Ranvier, in Pig, 616
- nose, in Chick, 433
- notochord, 64, 68; in Amphioxus, 92, 95; in Frog, 131, 134, 135, 135, 157, 177, 178, 180, 248; in Teleost, 269, 270, 270, 271, 272, 274; in Gymnophiona, 277, 277; in Chick, 305, 307, 310, 312, 313, 314, 325, 328, 329, 396, 397; in Mammal, 527, 528, 529; Pig, 569, 575, 655
- notochordal canal, in Mammal, 527
- nucleoli, chromatin, in Frog, 108, 109
- nucleus,
 of egg, 9, 43, 44, 45, 46; in Chick, 284, 285, 287; in Frog, 108, 109, 114, 115; in Mammal, 492, 493, 505
 of Pander, in Chick, 284, 286
 of sperm, 13, 505
 of Terni, in Chick, 414
- odontoblast, in Mammalian tooth, 658, 659, 660, 661
- odontoblast layer, in Mammalian tooth, 623
- oestrogens, in Mammal, 503
- oestrone (theelin), in Mammal, 499, 500, 501, 503
- oestrus cycle, in Mammal, 494, 495, 495, 498, 499
- oil vacuole, in Teleost egg, 263, 266, 267
- olfactory bulb or lobe, in Frog, 179; in Chick, 410, 412; in Pig, 610, 611
- olfactory capsules, in Frog, 250; in Chick, 440
- olfactory epithelium, in Chick, 390, 425, 425, 426
- olfactory nerve (See cranial nerve)
- olfactory organ, in Frog, 196, 197, 199; in Chick, 389, 425, 426; in Pig, 616
- olfactory pit, in Frog, 160, 160, 169, 170, 197; in Chick, 379, 390, 425, 426, 433, 435; in Pig, 564, 565, 566, 568, 573, 607, 609
- omental bursa, in Pig, 626, 629, 632
- omentum,
 great, in Pig, 626, 630
 lesser or gastro-hepatic, in Pig, 626, 630
- oocyte, 9; in Frog, 106, 107, 108, 109; in Chick, 284, 285, 473; in Mammal, 491, 492, 505 (See also egg)

- oögenesis, 8-11; in *Amphioxus*, 77; in Frog, 107, 108; in Chick, 283-286; in Mammal, 489-493
- oögonia, 9; in Frog, 106, 107; in Chick, 283, 284, 472; in Mammal, 489, 491
- operculum, in Frog, 172, 196, 202; in Chick ear, 425
- Old World Monkeys (*Rhesus*), sexual cycle in, 497, 503
- Opossum,
allantois of, 530, 532
implantation in, 532
placenta of, 530, 531
sperm of, 13
yolk-sac of, 530, 531, 532
- Oppenheimer, J. M., 267, 271
- optic chiasma, in Frog, 177, 177, 192; in Chick, 349, 411, 412; in Pig, 569, 611
- optic cup, in Frog, 190; in Chick, 353, 354, 354, 388, 417, 418; in Pig, 568, 573, 576
- optic lobes, in Frog, 181; in Chick, 411, 413; in Pig, 567
- optic nerve (*See* cranial nerves)
- optic recess, in Frog, 177, 177; in Chick, 348, 349, 349; in Pig, 569, 611
- optic stalk, in Frog, 159, 190, 191, 192; in Chick, 350, 353, 354, 419, 420; in Pig, 611
- optic thalami, in Pig, 612
- optic vesicle, in Frog, 159, 159, 165; in Chick, 230, 328, 333, 342, 349, 353, 354; in Pig, 564, 565, 567, 573
- ora serrata, in Chick eye, 417, 418
- oral cavity, in Chick, 371, 373, 435; in Pig, 564, 622
- oral evagination, in Frog, 159, 162, 165, 177, 189
- oral membrane or plate, in Frog, 178, 200; in Chick, 324, 348, 349, 384; in Pig, 574, 575
- oral mucous gland ("sucker"), in Frog, 150, 151, 161, 165, 170
- organ of Corti, in Mammalian ear, 618, 619
- organizer, 141
- organizer theory, evidence for, 138-144
- Ornithorhynchus*, extra-embryonic membranes and appendages in, 530, 531
- Orthoptera, synopsis in, 18
- osseous fibers, 240, 241, 660
- osteoblasts, 240, 241, 241, 242, 243, 244, 245, 246, 656
- osteoclasts, 242, 244, 245, 246
- ostium urogenitale, in Pig, 648, 652, 653, 654
- otocyst (*See* auditory vesicle or capsule)
- outer limiting membrane, of nerve cord, 568
- outer tubules of mesonephros, in Frog, 230, 231
- outer zone of metanephros, 468, 468, 474
- ovarian sacs, in Frog, 237
- ovary, 3, 4; in Frog, 106, 106; in Chick, 281, 282, 283, 472; in Mammal, 488, 489; Pig, 645, 649, 653
- ovigerous cords, 4; in Chick, 472, 473
- ovulation, in Frog, 110; in Chick, 287; in Mammal, 493, 495, 495, 501
- ovum (*See* egg)
- pachytene stage in meiosis, 17, 18, 20, 21, 37
- palatine process,
lateral, in Pig, 621, 622, 623
median, in Pig, 621, 622
- palato-quadrato cartilage (*See* cartilage)
- pallial layer, 10
- pancreas, 67; in Frog, 206; in Chick, 374, 375, 399, 445, 446, 447; in Pig, 568, 580, 580, 582, 631
- pancreatic acini, in Pig, 631
- pancreatic ducts, in Frog, 206; in Chick, 446; in Mammals, 632; in Pig, 631, 631
- papilla of feather germ, 435
- papillary muscles, in Pig, 642
- parabronchi, in Chick, 443, 444
- parachordal cartilages or plates (*See* cartilage)
- paradidymis, in Chick, 472; in Pig, 647, 649
- paraphysis, in Chick, 412
- parathyroids, in Chick, 443; in Pig, 625, 625
- parencephalon, in Chick brain, 383, 384, 410, 411
- Parker, G. H., 506, 510
- paröphoron, in Chick, 473
- pars basilaris, in Frog ear, 193, 195
- pars cavo-pulmonalis, in Chick, 450
- pars distalis of pituitary, in Mammal, 158, 612

- pars intermedia of pituitary, in Mammal, 158, 612
 pars tuberalis of pituitary, in Mammal, 158, 612
 parturition, stimulation for, 503, 504
 Pasteels, J., 137, 300, 303, 311
 Patten, B. M., 343, 459
 pecten, in Chick eye, 419, 419, 420, 420
 pectoral girdle, in Frog, 254; in Chick, 438; (shoulder in Pig), 656
 peduncle, in Pig, 612
 Pelagia noctiluca, loss of chromatin in, egg of, 26
 pelvic girdle, in Frog, 254; in Chick, 438; in Pig, 657
 pelvis, or pelvic portion, of kidney, in Pig, 605, 644
 penetration of sperm, 39, 40
 penetration path of sperm (*See* entrance path)
 penile raphe, in Pig, 654
 penis, in Mammal, 488; Pig, 645, 646, 652, 653
 Perameles,
 allantois in, 530, 532
 implantation in, 532, 533
 placenta in, 533, 533
 yolk-sac in, 530, 532
 perforatorium, 13
 periblast (central or subgerminal, and marginal), in Teleost, 264, 264, 265, 265, 270, 271, 271, 274; in Chick, 293, 294, 295, 296, 297
 periblast nuclei, in Chick, 297
 pericardial cavity, in Frog, 165, 167, 177, 189, 203, 211, 211, 215; in Chick, 326, 339, 341, 381; in Mammal, Rabbit, 516; Pig, 578, 579, 633, 634
 pericardium, in Frog, 157, 167; in Chick, 341, 466; in Pig, 633, 634
 perichondrium, 244, 656
 perichordal sheath, 396, 397
 perilymphatic fluid, in Frog, 195; in Chick, 423
 perilymphatic space, in Frog, 195; in Chick, 423; in Pig, 619
 perineum, in Pig, 653
 periosteum, 242, 246, 246, 247, 252, 254, 439, 623, 656
 peritoneal cavity, in Chick, 465, 466 (*See also* coelom)
 peritoneal epithelium, in Mammal, 490, 491
 peritoneum, in Frog, 215; in Chick, 466
 perivitelline membrane, in Amphioxus egg, 78
 perivitelline space, 40; in Frog, 114; in Teleost, 263; in Chick, 286; in Mammal, 493
 Peter, K., 300, 303
 Peter's blastocyst, in Mammal, 552, 552
 pharyngeal region, in Frog, 157; in Chick, 335, 336, 372, 398, 412
 pharynx, in Frog, 162; in Chick, 373, 384; in Pig, 566, 569, 576, 577, 624, 625
 Phillips, R. E., 289
 Phillips, R. W., 506
 Piatt, J., 386
 Pig,
 allantois in, 534, 537
 amnion formation in, 515
 blastoderm, 515, 522, 524, 525, 527
 blastodermic vesicle (blastocyst), 254, 535, 535, 537
 cleavage in, 508
 gastrulation in, 510-513
 implantation in, 535, 536
 later development of, 561-654
 oestrus cycle in, 494, 495, 496
 placenta in, 534-537
 reasons for study of, 486, 487
 yolk-sac in, 513, 535
 pigment, in Frog egg, 109, 117, 118, 120
 pigmented layer of retina or optic cup, in Frog, 189, 190, 191, 192; in Chick, 354, 417, 418
 pineal gland, in Chick, 412 (*See also* epiphysis)
 pituitary, in Frog. origin and nomenclature of parts, 157, 158, 159
 anterior, in Chick, 335, 371, 384, 410, 412; in Pig, 577, 610, 611
 effect on metamorphosis, in Amphibia, 174
 effect on sex cycle, in Mammal, 501, 502
 posterior, in Chick, 371, 372
 placenta, in Mammals; Marsupials, 531-533, 533; Ungulates, 534-537, 534, 535; Carnivores, 538, 538, 539, 540; Rodents, 543, 544, 545; in Primates, 550-560, 554, 555
 deciduate, 540, 545, 560
 discoidal, 545
 indeciduate, 537

- source of oestrogens, 502, 503
- zonary, 539
- placodes, in Chick, 352, 353, 415, 416; in Frog, 160, 160, 184, 185, 186, 187, 201
- plectrum collumella, in Frog, 196
- pleura,
 - parietal, in Pig, 633
 - visceral, in Pig, 632
- pleural cavity, in Chick, 466; in Pig, 632, 633, 634
- pleuro-pericardial folds, in Pig, 634
- pleuro-pericardial septum in Pig, 633, 634
- pleuro-peritoneal folds, in Pig, 634
- pleuro-peritoneal septum, in Chick, 466; in Pig, 633
- plica encephal ventralis, in Chick brain, 411
- Pohlman, A., 458, 460
- polar bodies, 27, 28, 29, 41, 44, 45; in Amphioxus, 77, 78, 80; in Frog, 108, 111, 111, 114; in Chick, 287; in Mammal, 505, 505
- polyoestrus, Mammals, 496
- polyspermy, 39; in Frog, 114; in Chick, 287
- pons Varolii, in Chick, 413; in Pig, 613
- pontine flexure, in Chick, 409, 411; in Pig, 613
- postanal gut, in Frog, 207; in Chick, 375, 376, 377; in Pig, 583
- postbranchial bodies, in Chick, 442, 443; in Pig, 625, 625
- posterior chamber, of eye, in Frog, 190, 191; in Chick, 354, 390, 418
- postganglionic fibers of sympathetic system, in Chick, 387, 414, 416
- post-reduction, 20, 20, 21, 22, 23, 24
- preganglionic fibers of sympathetic system, in Chick, 387, 414, 416
- pregnancy, 498, 502
 - tests for, 503
- premaxillary region, in Pig, 608, 622
- preoral gut, in Chick, 372; in Pig, 576
- preoral pit, in Amphioxus, 93, 101
- prepuce, in Pig, 652, 654
- pre-reduction, 20, 20, 21, 21, 22, 24
- Prichard, M. M. L., 458
- primary (elastic) sheath of notochord, in Frog, 208
- Primates,
 - allantois in, 546, 546, 547, 548
 - implantation in, 550-560
 - placenta in, 550-560
 - sexual cycle in, 496-503
 - yolk-sac in, 546, 546, 547, 549, 549, 550, 551, 552, 557
- primitive folds, in Chick, 305, 305, 306, 307
- primitive groove, in Chick, 304, 305, 306, 307, 321, 325
 - in Mammal; in Pig, 524, 526, 528
- primitive knot, or Hensen's knot, in Chick, 305, 305, 306, 307, 309, 310; in Mammal; Pig, 523, 524, 525, 526, 528
- primitive pit, in Chick, 305, 305, 307, 309, 315; in Mammal, Pig, 529
- primitive plate, in Chick, 305, 306
- primitive streak, 65, 66; in Frog, 152, 153; in Teleost, 269, 270; in Gymnophiona, 275; in Chick, 301, 302, 304, 305, 306, 308, 308, 309, 311, 313, 314, 315, 320, 328, 333; in Mammal, 513, 522, 523, 525, 525, 526, 526, 527, 528, 529, 562
- primordial germ cells, 4, 6, 7; in Chick, 469, 470, 471, 472; in Mammal, 470, 489, 491
- proamnion, in Chick, 305, 317, 320, 321; in Mammal; Rabbit, 516; Pig, 529, 574
- processus vaginalis, in Pig, 646, 650, 651
- proctodael pit, or proctodaeum, in Frog, 152, 153, 154, 157; in Chick, 448, 449, 449; in Pig, 653, 583
- progesterone, in Mammal, 499, 500, 501, 502, 503
- prolactin, in Mammal, 500
- Prolan A and B, in Man, 503
- proliferation, origin of mesoderm by, 65, 66; in Chick, 309, 317; in Pig, 527
- pronephric capsule, in Frog, 227
- pronephric chamber, in Frog, 228, 229
- pronephric duct (also segmentation or Wolffian), in Frog, 201, 227, 228, 229; in Chick, 356; in Pig, 603
- pronephric swelling, in Frog, 150
- pronephric tubules, in Frog, 226, 226, 227; in Chick, 355, 356; in Pig, 603
- pronephros, in Frog, 155, 164, 167, 227, 227; in Chick, 355, 391; in Pig, 603
- pronuclei (See nucleus, of egg, of sperm)
- pro-oestrus, in Mammal, 494, 495, 495, 498
- prophases in meiosis, 18-24

- prosencephalon, in Frog, 156, 157, 177, 177; in Chick, 348, 348-350, 383, 409-412; in Pig, 565, 567
- prostate glands, in Mammal, 488; in Pig, 645, 646, 648
- proventriculus, in Chick, 446
- pseudopregnancy, 494, 495, 502
- pubis, in Chick, 438; in Pig, 657
- pulmo-enteric recess, in Chick, 381
- pulp cavity in tooth, 659, 660
- pupil of eye, in Frog, 190; in Chick, 350
- pygostyle, in Chick, 438
- Quirring, D. P., 450
- Rabbit,
- allantois in, 516, 542, 543
 - amion in, 514, 515, 516, 517
 - blastoderm, 514, 515
 - blastodermic vesicle in, 510
 - cleavage in, 506
 - embryonic knob in, 514, 515
 - implantation in, 542, 543
 - maturation or meiosis in egg, 506
 - mesoderm formation in, 515, 516
 - movement of egg in oviduct in, 510
 - ovulation in, 493
 - placenta in, 542, 543
 - sperm in oviduct of, 506
 - yolk-sac in, 513, 516, 542, 543, 545
- radius, in Pig, 652
- ramus communicans or rami communicantes, in Frog, 189; in Chick, 387, 414, 414, 415; in Pig, 572
- Randles, C. A., 366
- raphe, penile, in Pig, 652, 654
- Rat,
- corpora lutea in, 502
 - descent of testes in, 651
 - fertilization of egg, 508
 - movement of sperm in, 507, 508
 - sex cycle in, 496
 - spermatogenesis in, 15
- Rathke's pocket, in Chick, 335, 349, 371, 372, 372, 373, 384; in Pig, 568, 569, 577, 611, 612, 625
- Rawles, M. E., 300, 311, 312
- recessus opticus, in Chick, 410, 411, 412 (*See also* optic recess)
- rectal evagination, in Frog, 157
- rectum, in Frog, 105, 207; in Chick, 282, 368, 400, 445, 448, 448, 449, 449; in Pig, 581, 584, 630, 645, 646, 647, 648, 653
- reductional division, 19, 20, 21, 22, 24
- rejuvenescence, 47
- relaxin, 501
- renal capsule, in Frog, 232
- reproduction, 47
- reproductive organs or system, in Frog, 105, 106; in Chick, 280-284, 282; in Mammal, 488-489
- respiratory system, in Frog, 206; in Chick, 337, 398, 399, 443-445; in Pig, 577, 579, 632
- sources of, 67
- rete cords, in Frog, 236, 236, 237; in Chick, 470, 471, 471, 472
- retina or retinal layer, in Frog, 189, 190, 191, 192; in Chick, 354, 417, 418; in Pig, 617
- retinal zone, in Chick eye, 417
- revitalization through conjugation, 48
- rhinencephalon, in Pig, 611
- rhombencephalon, in Frog, 156, 157, 177, 178, 180, 181; in Chick, 348, 351, 384, 384, 413; in Pig, 567, 612
- Rhumbler, 58
- ribonucleoprotein, in Frog, 109
- ribs, in Chick, 437; in Pig, 656
- Rock, J., 553
- rods and cones in eye of Frog, 192
- Romanoff, A. L., 306
- root,
- of hair, 663
 - of tooth, 660, 661, 662
- root sheath, inner and outer, of hair, 662, 663, 664
- rotation of Frog egg during gastrulation, 129, 129
- round ligament,
- of liver, 638
 - of uterus and ovary, 647, 653
- Roux, W., 121
- Rudnick, D., 300, 311
- Rugh, R., 110
- rutting periods, in Mammal, 504
- Sabatier, 455, 458
- saccule, in Frog, 193, 194; in Chick, 389
- sacculus, in Pig, 617, 618, 618, 619
- scala,
- tympani, in Mammal, 618, 619
 - vestibuli, in Mammal, 618, 618
- scale, in Chick, 436
- scapula, in Chick, 438; in Pig, 656
- Schechtman, A. M., 126, 132, 133
- Schott, R. G., 506

- Schotté, O. E., 161
 Schultze, O., 121
 Schwind, J. L., 174
 sclerotic coat, in Frog, 192; in Chick, 418
 sclerotomal cells, in Frog, 209
 sclerotome, 64, 69; in *Amphioxus*, 99, 100; in Frog, 166; in Chick, 329, 335, 371, 396, 397; in Pig, 584, 585
 Scott, H. M., 289, 291
 scrotal ligament, in Pig, 646, 650
 scrotal raphe, in Pig, 652, 654
 scrotal sac, or scrotum, in Mammal, 488; Pig, 646, 650, 651, 652, 654
 Sea Bass (*Serranus*), 264, 266, 266
 sebaceous glands, in Mammal, 663, 664
 secondary or fibrous sheath of notochord, in Frog, 208
 Seesell's pocket, in Pig, 568, 569, 577, 625
 Segal, S. J., 233
 segmental (vertebral) plate, in Frog, 165; in Chick, 324
 segmentation or cleavage, 50, 51, 52, 53; in *Amphioxus*, 83-87, 85; in Frog, 117, 123, 124, 124, 125; in Teleost, 262-264, 264; in *Gymnophiona*, 273; in Chick, 292-299, 293; in Mammal, 508, 509, 510
 accessory, in Chick, 293, 294
 cavity (blastocoel), in Teleost, 263, 264 (*See also* blastocoel)
 holoblastic or total, 52
 mereoblastic or discoidal, 53; in Teleost, 262, 264
 unequal, 53
 semen, in Mammal, 488
 sperm per c.c. in, 507
 semicircular canals, in Frog, 193, 194; in Chick, 389, 422, 422; in Pig, 617, 618
 semilunar valves, in Chick, 451; in Pig, 642
 seminal vesicles, in Frog, 105, 106; in Mammal, 488; Pig, 645, 646, 648
 seminiferous tubules, in Frog, 105, 238; in Chick, 281, 471, 471, 472; in Mammal, 488
 sense organs, early development, in Frog, 159
 sense plate, in Frog, 148, 149, 150, 151
 septa, in Frog heart, 213, 214; in Chick, 402, 403, 450; in Pig, 588, 588, 589, 589, 641, 641, 642
 septum,
 primum in Pig heart, 588, 589, 589, 641, 642, 643
 secundum, in Pig heart, 588, 589, 589, 641, 642, 643
 spurium, in Pig heart, 589
 transversum, in Frog, 215; in Chick, 465; in Pig, 579, 633, 633, 634
 sero-amniotic connection, in Chick, 359, 360, 361, 364, 365, 366; in Mammal, Rabbit, Pig, 517
 serosa, in Chick, 360
 serous membrane of uterus, in Mammal, 489
 Sertoli cells, 6, 15, 15; in Frog, 105; in Chick, 471
 Severinghaus, A. E., 503
 sex-cell cord, in Frog, 235, 235
 sex-cell ridge, in Frog, 235, 235
 sex chromosomes, 30-37, 30, 31, 32, 33, 34, 35, 36
 sex determination, 38
 sex reversal, in Amphibia, 238-240
 sexual cords, 5, 5; in Chick, 469, 471, 471, 472, 473
 sexual cycle, in Mammals, female, non-Primates, 493-496, 495; Primates (menstrual), 495, 496-498; male, non-Primates, 504
 anovulatory, in Primates, 495, 499-500
 causes of, 499-501
 functions of, 501, 503
 Sheep,
 inner cell mass in, 510
 movements of sperm in, 506, 507
 sexual cycle in, 496
 shell membrane, in Chick, 282, 286, 288, 290
 sinus,
 rhomboidalis, in Pig, 562
 terminalis, in Chick, 317, 322, 346, 347, 408, 409; in Mammal, Marsupials, 530
 venosus, in Frog, 178, 212, 213; in Chick, 343, 345, 348, 349, 381, 384; in Pig, 536, 587, 589, 597, 598, 600, 639, 640, 642, 643
 skeletogenous sheath, in *Amphioxus*, 100; in Frog, 247
 skeleton, 67; in Frog, 240-254; in Chick, 436-441; in Pig, 655-658
 appendicular, in Frog, 252-254; in Chick, 438-440; in Pig, 656-658

- skin, dermis and epidermis, 67
 skull bones, in Frog, 248-251; in Chick, 440-441; in Pig, 655
 alisphenoids, in Chick, 441
 angulars, in Chick, 441
 basisphenoid, in Chick, 441
 dentals, in Chick, 441
 epiotic, in Chick, 441
 ethmoid, in Pig, 655
 exoccipital, in Frog, 249
 frontals, in Chick, 441
 fronto-parietals, in Frog, 249, 251
 hyoid apparatus, in Chick, 441
 internasal septum, in Chick, 441
 interorbital, 441
 jugals, in Chick, 441
 lachrymals, in Chick, 441
 maxillae or maxillary, in Frog, 249; in Chick, 441
 nasals, in Frog, 249; in Chick, 441
 naso-turbinals, in Pig, 655
 occipital, in Pig, 655
 opercular, in Chick, 441
 opisthotic, in Chick, 441
 orbitosphenoid, in Chick, 441
 palatine, in Chick, 441
 parasphenoid, in Chick, 441
 parietals, in Chick, 441
 periotics, in Pig, 655
 premaxillary and premaxillae, in Frog, 249; in Chick, 441
 ptotic, in Chick, 441
 pterygoid, in Frog, 249; in Chick, 441
 quadrate, in Chick, 441; in Pig, 655
 quadrato-jugal, in Frog, 249; in Chick, 441
 sphenoids, in Pig, 655
 squamosals, in Chick, 441
 supra-angulars, in Chick, 441
 supra-occipitals, in Chick, 441
 vomer, in Chick, 441
 snout of Pig, 609
 Soderwall, A. L., 507
 somatic cells, 3
 somatopleure, in Frog, 165; in Chick, 326, 397 (See also somatic mesoderm)
 somite, 69; in Amphioxus, 92, 93, 95, 96, 98; in Frog, 165, 166, 166, 208; in Teleost, 274; in Chick, 306, 322, 324, 325, 325, 328, 329, 333, 334, 334, 335, 370, 371, 379, 395, 396, 396; in Pig, 525, 564, 565, 575, 584
 Sonneborn, T. M., 48
 spawning, in Amphioxus, 78; in Frog, 111
 Spemann, H., 139, 140, 143
 sperm,
 development of, 14
 ducts for, in Frog, 106
 entrance point plane of, in Frog egg, 115, 116
 motility of, in genital tract, of Mammals, 506, 507, 508
 penetration of egg by, 39
 survival time of, in genital tracts of Mammals, 507, 508
 varieties of, 13
 spermatids, 14, 16
 spermatocytes, 14, 15, 16, 18, 19, 23, 29; Chick, 472
 spermatogonia, 5, 14; in Chick, 471, 472
 spermatozoa, 11, 12, 13; in Frog, 105 (See also sperm)
 spinal cord, in Frog, 181, 182; in Chick, 351, 384, 385; in Pig, 570, 613, 614 (See also neural tube and nerve cord)
 spiracle, in Frog, 170, 172
 spireme, 16
 splanchnocoel, in Amphioxus, 99, 99; in Frog, 210 (See also coelom)
 splanchnocranium, in Chick, 441
 splanchnopleure, in Frog, 165; in Chick, 326; in Pig, 573 (See also mesoderm splanchnic)
 spleen, in Frog, 216, 225; in Chick, 399; in Pig, 626, 629
 spongioblasts, in Pig, 567, 570, 614
 Spratt, N. T., 301, 303, 305, 308, 309, 310, 311, 313
 Stanley, L. J., 469
 stapes, 424; in Pig, 618, 619, 620
 Stellate cells, in Pig, 658
 sternum, in Chick, 437; in Pig, 656
 stigmata, in Chick, 282
 stomach, in Frog, 207; in Chick, 372, 373, 399, 445; in Pig, 568, 579, 579, 580, 626, 627, 628, 631
 stomodaeal invagination, in Frog, 150, 151
 stomodaeum, in Frog, 157, 169, 170, 200; in Chick, 335; in Pig, 574, 576, 622
 Straus, W. L., 397, 437
 Streeter, G. L., 552
 strepsinema, 19

- stroma, 4; in Frog, 107; in Chick, 283, 471, 472
- stylohyal, in Chick, 425
- styloid process, in Pig, 624
- subgerminal cavity, in Chick, 294, 297, 303; in Mammal, 510
- subzonal layer, in Mammal, 509, 510, 513
- sulcus limitans, in Pig, 613
- sulcus rhinalis, in Pig, 610, 611
- summary, first day of Chick, 330-331; second day of Chick, 367-369; third day of Chick, 391-394; fourth day of Chick, 428-432; fifth day of Chick, 476-479
- superfetation, 504
- supernumerary nuclei (*See* merocytes)
- Swift, C. H., 469
- Swingle, W. W., 238
- sympathoblasts, in Frog, 233
- synapsis, 17
- synaptene stage, 16, 17, 18, 20
- syncytium, in Teleost, 264
- synencephalon, in Chick, 383, 384, 410
- synizesis, 17, 18, 20
- tail, in Mouse, 508
- tail, of sperm, 12, 12
- tail bud, in Chick, 338, 375, 376, 377; in Pig, 564
- tail fold, in Chick, 338, 338
- tarsals, in Chick, 439
- Tarsius*,
allantois in, 551
amnion in, 550, 551
implantation in, 550
placenta in, 550, 551, 551
yolk-sac in, 551
- tarso-metatarsals, in Chick, 439
- Teacher, J. H., 552
- tectoral membrane, in Pig, 618, 619
- teeth, in Frog, 200; in Mammals, 623, 624, 658-662, 658
dentine in, 67, 658, 659
enamel and enamel organ in, 67, 658, 658
- telencephalon, in Frog, 179, 180; in Chick, 333, 349, 350, 383, 384, 409, 410, 411; in Pig, 567, 568, 610
- teleolecithal eggs, 10; in Frog, 109
- temperature effect on Frog egg, 113
- tendons, connections with bone, 247
- tertiary egg coverings, in Frog, 111, 111, 112; in Chick, 286, 288, 289
- testis, 3, 5, 5; in *Amphioxus*, 76; in Frog, 104, 105, 105; in Chick, 280, 281, 468-472, 471; in Mammal, 488; Pig, 645, 645, 646, 646, 650, 651
appendix of, in Pig, 649
descent of, in Pig, 646, 650, 651; in Rat, 651
effect of retention, 651
- testosterone, use in sex reversal, 239
- tetrads, in meiosis, 18-25, 19, 24
- thalamus, in Chick, 441
- theca of ovarian follicle, in Chick, 283
externa, in Frog, 107; in Chick, 280; in Mammal, 491
interna, in Frog, 107; in Chick, 280; in Mammal, 491
- thymus, 67; in Frog, 203, 204, 205, 205; in Chick, 442, 442, 443; in Pig, 624, 625, 625
- thyroid, 67; in Frog, 178, 205, 205; in Chick, 336, 349, 372, 373, 384, 398, 442; in Pig, 568, 625, 625
effect on metamorphosis, in Frog, 174
- tibia, in Chick, 439; in Pig, 658
- Ting, H. P., 125
- Tomes fibers, in Mammalian tooth, 658, 660
- Tomes' processes, in Mammalian tooth, 658, 661
- tongue, in Frog, 200; in Chick, 398; in Pig, 621, 623, 623
- tonsils, in Pig, 622, 624, 625
- torus transversus, in Frog, 177, 177; in Chick, 383, 384, 411, 412
- Towns, P. L., 144
- trabeculae carneae of heart, in Pig, 588, 593, 641, 642
- trabeculae of bone, 243, 244, 246
- trabeculae of cartilage (*See* cartilage)
- trachea, in Chick, 337, 398, 443; in Pig, 568, 578, 579
- transverse neural ridge or fold, in Frog, 136, 148
- transverse (or costal) processes, in Frog, 247; in Chick, 437; in Pig, 656
- triblastic, definition of, 63
- trigeminal ganglion (*See* ganglia)
- trigeminal nerve (*See* nerve, mandibular and maxillary, *also* ophthalmic)
- Triton, pregastrular map of formative materials in, 138

- trophoblast, in Mammal, 503, 508, 509,
 510, 512, 513, 514, 514, 515, 515,
 516, 518, 518, 519, 519, 520, 521,
 530, 532, 533, 548, 549, 549, 550,
 553
 allantoidean, 542
 omphaloidean, 520, 541, 543, 544
 trophoderm, in Mammal, 516, 526, 542,
 544, 545, 552, 554, 555, 556, 557,
 558, 559
 allantoidean, 516, 519, 520, 541, 542
 truncus arteriosus, in Frog, 177, 178,
 212, 214; in Chick, 341, 342, 342,
 378, 404, 451, 452, 453, 454; in
 Pig, 536, 569, 587, 588, 593, 594,
 636, 642
 tubal ridges, in Chick, 428
 tuberculum,
 impar, in Pig, 623
 mamillare, in Chick, 412
 posterius, in Frog, 156, 157, 177, 178,
 180; in Chick, 349, 349, 410, 412;
 in Pig, 569
 tubo-tympanic cavity (*See* middle ear)
 tubules of kidney, in Pig, 644 (*See also*
 mesonephric tubules and meta-
 nephric tubules)
 tunica albuginea, in Frog, 105; in
 Chick (*See* albuginea)
 tunica vaginalis, in Pig, 646, 651
Tupaija javanica,
 anion in, 512, 514
 blastodermic vesicle in, 512
 inner cell mass in, 512
 twins, in Frog, 121
 tympanic cavity, in Frog, 195; in
 Chick, *see* middle ear; in Pig, 620
 tympanic membrane, in Frog, 195; in
 Chick, *see* tympanum; in Pig, 618,
 620
 tympanum, in Chick, 424

 Uhlenhuth, E., 174
 ulna, in Pig, 656
 ultimo-branchial (suprapericardial),
 bodies, in Frog, 204, 205, 205; in
 Chick (postbranchial), 442, 443;
 in Pig (postbranchial), 625, 625
 umbilical cord or stalk, in Mammal,
 Rodent, 541; Primates, 546, 547,
 548, 550, 556; Pig, 535, 565, 573,
 606, 607, 645
 umbilical stalk, in Chick, 400, 445,
 447

 umbilicus,
 somatic, in Chick, 361, 362; in Pig,
 573
 yolk-sac, in Chick, 362, 365; in Pig,
 573
 Ungulates,
 allantois in, 534, 536
 blastodermic vesicle in, 510, 511,
 535
 implantation in, 535, 536, 537
 placenta in, 534, 535, 536, 537
 yolk-sac in, 534
 unipolar ingression, in Triturus, 132
 ureter, in Frog, 105, 106, 106; in Chick,
 427, 467, 468, 468; in Pig, 604,
 605, 605, 644, 645, 646, 647, 648,
 649, 651
 urethra in Mammal, 488; in Pig, 645,
 646, 647, 648
 penile, in Pig, 654
 prostatic, in Pig, 654
 urinary bladder, in Frog, 105, 208; in
 Pig, 645, 646, 647, 648, 649
 homologue of, in Chick, 365
 urinogenital or urogenital ducts, in
 Frog, 233, 234, 234, 235; in Chick,
 390, 391, 427, 428, 473, 474, 474;
 in Pig, 646-649
 urinogenital or urogenital sinus, in Pig,
 584, 604, 645, 647, 648, 649, 653,
 654
 urinogenital or urogenital system, 67;
 in Frog, 225-240; in Chick, 355-
 357, 391, 426-428, 466-475; in Pig,
 602-605, 643-654
 urodaeum, in Chick, 449, 449
 Urodele, gastrulation in, 137, 137, 139
 uro-rectal fold, in Pig, 584, 645, 647,
 653
 urostyle in Frog, 248
 uterine endometrium, 489, 494, 500;
 in Ungulates, 535, 537
 uterine epithelium, Ungulates 536, 537;
 Carnivores, 538; Rodents, 540,
 541, 542, 543; Primates, 550, 551,
 552, 553, 555, 556, 557
 uterine glands, in Man and Apes, 552,
 554, 555
 uterine mucosa, Carnivores, 538; Ro-
 dents, 543; Man and Apes, 555,
 556, 557, 558, 559
 uterine secretions ("milk"), in Marsu-
 pials, 532; Ungulates, 534; Carni-
 vores, 538

- uterus or uteri, in Frog, 105, 107; in Chick, 282, 283; in Mammal, 534, 541, 542, 543, 553, 556, 556, 557; in Pig, 645, 647, 649
- bicornis, in Mammal, 489
- duplex, in Mammal, 489
- masculus, in Pig, 649
- products of and time spent in, in Chick, 289, 290
- simplex, in Mammal, 489; Pig, 649
- utricle, in Frog, 193, 194; in Chick, 389, 422, 422; in Pig, 617, 618, 618, 619
- vagina, in Chick, 282, 283; in Mammal, 489; Pig, 645, 647, 649, 654
- vagus (See ganglion and nerve)
- valves of heart, in Pig, mitral or bicuspid, 641, 642 tricuspid, 641, 642
- valvulae venosae, in Pig, 578, 589, 589, 596, 642, 643
- variation, causes of, 48
- vas deferens, or vasa deferentia, in Frog, 105, 106, 233; in Chick, 281, 428, 471, 473; in Mammal, 488; Pig, 645, 646, 646, 648, 651
- vasa efferentia, in Frog, 105, 106, 233; in Chick, 281, 471
- vegetal pole of egg, 8, 10, 55; in Amphioxus, 79, 80, 82, 84; in Frog, 109, 117, 129; in Fish, 262
- vein or veins, abdominal, in Frog, 224 anterior cardinals, in Frog, 220, 221, 221; in Chick, 345, 346, 380, 382, 406, 463; in Pig, 536, 576, 587, 589, 596, 597, 598, 599, 601, 638, 639 azygos, in Pig, 599, 640 caudal, in Frog, 222, 223; in Chick, 461, 465 cervico-thoracic, in Pig, 599, 640 femoral, in Frog, 223, 224 gonadal, in Pig, 599 hepatic, in Frog, 218, 220, 221; in Chick, 405, 462; in Pig, 596, 597, 600 hepatic portal, 220, 221, 223; in Chick, 405, 462; in Pig, 581, 596, 597, 600, 637, 638 iliac (common, external, internal), in Frog, 223, 224; in Chick, 464, 465; in Pig, 598, 599, 637, 639, 640, 797 innominate, in Frog, 221, 222; in Pig, 599, 639 intermediate, in Chick, 383, 408, 409 intersegmental, in Pig, 601 intestinal, in Pig, 596 jugular (external, internal), in Frog, 203, 221, 221, 222; in Chick, 373, 379, 380, 461; in Pig, 596, 599, 601, 638, 639 median cardinal, in Frog, 222, 223 mesenteric, in Chick, 461, 461, 463; in Pig, 599 omphalomesenteric (See vitelline) pelvic, in Frog, 221, 223 posterior cardinal, in Frog, 220, 221, 221, 228, 235; in Chick, 329, 345, 346, 356, 380, 380, 381, 382, 390, 407, 462, 463, 464, 464, 465; in Pig, 536, 581, 582, 583, 587, 589, 596, 597, 598, 599, 601, 602, 605, 638, 639, 640 pulmonary, in Frog, 218, 224; in Chick, 408, 457; in Pig, 577, 603, 640, 641 renal, in Frog, 224; in Chick, 464, 464; in Pig (unlabeled), 599 renal portal, in Frog, 221, 223, 224; in Chick, 462; in Pig, 639 sciatic, in Frog, 221, 223, 224 subcardinal, in Chick, 380, 406, 407, 461, 462, 463, 464, 474; in Pig, 569, 582, 583, 596, 598, 599, 601, 602, 639 subclavian, in Frog, 221, 221; in Chick, 462, 463; in Pig, 578, 598, 599, 601, 638, 639 subscapular, in Frog, 221, 222; in Chick, 381 supracardinals, in Pig, 599, 640 umbilical, in Chick, 379, 381, 382, 405, 408, 457, 461; in Pig, 536, 569, 579, 581, 582, 583, 587, 596, 597, 598, 600, 638 ventral, of mesonephros, in Pig, 581, 582, 583, 598, 599, 603, 605 vitelline, in Frog, 213, 228; in Chick, 328, 339, 340, 342, 344, 345, 346, 347, 379, 381, 382, 383, 405, 406, 408, 409, 457, 460, 461; in Pig, 569, 579, 582, 587, 596, 597, 597, 598, 599, 600, 637 velar plates, in Frog, 202, 203, 204 velum transversum, in Chick, 349, 350, 410 vena cava, anterior or superior, in Frog, 221,

- vena cava,
 222; in Chick, 457, 461, 463; in
 Fig, 639, 641, 641
 posterior or inferior, in Frog, 218,
 221, 222, 232; in Chick, 406, 407,
 457, 461, 463, 463, 464, 464; in
 Fig, 569, 578, 579, 581, 588, 596,
 597, 598, 599, 602, 603, 638, 639,
 640
 vena intervertebral, in Chick, 464
 veno's ring about gut, in Chick, 405,
 406
 venous system, diagram of develop-
 ment of, in Fig, 598, 599
 ventral horns in nerve cord, in Chick,
 385; in Fig, 568, 570
 ventral mesentery, in Fig, 574
 ventricle or ventricles of,
 brain, in Frog, 177, 179, 181; in
 Chick, 350; in Fig, (III) 611, (IV)
 613
 heart, in Frog, 212, 214; in Chick,
 333, 341, 342, 342, 377, 378, 379,
 384, 402, 403, 445, 456, 457; in
 Fig, 569, 578, 579, 587, 588, 588,
 641, 641
 vermiform appendix, in Man, 629
 vermis of brain, in Fig, 613
 vertebra or vertebrae, 69; in Frog, 247,
 247; in Chick, 397, 436, 437, 437,
 438; in Fig, 655
 vertebral arch, in Chick, 437
 vertebral plates (segmental), 64, 69
 vestibule, in Fig, 645, 647, 654
 villi, in Fig, 536; in Carnivores, 539,
 540; in Man and Apes, 546, 547,
 549, 553, 554, 555, 556, 557,
 559
 visceral arches, in Frog, 250, 251, 251;
 in Chick, 335, 336, 372, 373, 398,
 403, 435, 441, 442, 442, 443; in
 Mammals, Man, 559; in Fig, 563,
 565, 566, 568, 576, 577, 593, 624
 (See also branchial arches)
 visceral clefts or furrows, in Chick, 336,
 372, 372, 373, 398, 442, 442; in
 Fig, 563, 564, 565, 576, 579 (See
 also branchial clefts)
 visceral pouches, in Chick, 335, 336,
 372, 372, 373, 398, 442, 442, 443;
 in Fig, 624, 625, 625
 visceral skeleton, in Frog, 251, 251; in
 Chick, 441
 vitelline membrane, of egg, 71; in Am-
 phioxus, 77; in Frog, 109, 111; in
 Chick, 361
 vitreous chamber, in Frog, 190
 vitreous humor, in Frog, 192; in Chick,
 418; chamber, 354, 380, 418
 Vogt, W., 136
 Von Baer, 280
 vulva, in Fig, 654

 Wang, W. H., 453
 Watterson, R. L., 437
 Weisman, A., 7, 48
 Werner (Stieve) blastocyst, 548, 552
 Wetzel, R., 311
 Whale, size of egg in, 493
 white matter of nerve cord, in Frog,
 181, 182; in Chick, 385; in Fig, 614
 Whitehead, W. H., 458
 Wilder, H. H., 194
 Wilens, S., 168
 Wilson, E. B., 75
 Wilson, H. V., 266
 Wimsatt, W. A., 508
 Windle, W. F., 458, 459
 wing-bud, in Chick, 379
 Winiwarter, H., 352
 Wislocki, G. B., 503
 Witschi, E., 196, 236, 238, 239, 468
 Wittek, M., 109
 Woodruff, 48
 Woodside, G. L., 300, 315
 Wolff, C. F., 280
 Wolffian duct (pronephric or meso-
 nephric), in Frog, 231, 233, 235; in
 Teleost, 274; in Chick, 329, 356,
 390, 391, 448, 449, 466, 466, 467,
 468, 470, 474, 474; in Fig, 568, 604,
 605, 645, 646, 647, 648, 649

 X-chromosome, 32, 32, 33, 36
zenopus capensis, section of notochord
 in, 247

 Y-chromosome, 30, 34, 36
 Yntema, C. L., 186, 352, 387
 yolk, 39,
 blastopore, in Chick, 318, 320, 362
 in egg of, Frog, 109, 122; Teleost,
 274; Chick, 284, 285, 286, 286, 287
 nuclei, in Teleost, 263; in Chick, 285
 nucleus complex, 40
 plug, in Frog, 130, 131, 132; in Gym-
 nophiona, 274, 275

white, in Chick, 284, 286
yellow, in Chick, 284, 286
yolk nuclei, in Frog egg, 109
yolk-sac, 61; in Chick, 319, 361, 362,
364, 365, 366, 448, 457, 475, 476;
in Mammal, 512, 529; Monotremes, 530, 531; Marsupials, 530,
531, 532; Ungulates (Pig), 513,
516, 534, 535, 536, 563, 564, 569,
575, 575, 586; Insectivores, 512,
518; Carnivores, 513, 537, 538,
538; Rodents, 512, 513, 516, 518,
519, 520, 540, 541, 542, 543, 545;
Primates (Man), 546, 547, 549,
550, 552, 557

endoderm (*See* endoderm)
septa, in Chick, 362, 364, 365
umbilicus, in Chick, 362, 364, 365,
366
yolk-stalk, in Chick, 362, 445, 447; in
Pig, 568, 573, 582, 627, 629
Young, W. C., 507, 508
zona pellucida or radiata, in Chick,
264; in Mammal, 492, 493, 506,
509, 510
zone of junction, in Chick, 294, 297,
298, 302, 322
Zwilling, E., 161, 194, 376
zygopophysis, in Chick, 437

